

IgG Memory B Cell Antibodies in Patients with Systemic Lupus Erythematosus

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SUMMARY (German)

Die beständige Autoantikörperproduktion in Patienten mit Systemischem Lupus Erythematoses (SLE) suggeriert die Existenz eines autoreaktiven humoralen Gedächtnisses; die Häufigkeit selbstreaktiver Gedächtnis-B-Zellen in SLE wurde jedoch noch nicht ermittelt. Unter normalen Umständen exprimieren neu gebildete B-Zellen im Knochenmark Autoantikörper mit hoher Frequenz, darunter auch antinukleäre Antikörper. Diese autoreaktiven B Zellen unterliegen, bevor sie zu naiven immun-kompeten Zellen heranreifen, einer strengen Überwachung an zwei Kontrollpunkten für Selbsttoleranz, einer im Knochenmark und einer in der Peripherie. Im Gegensatz dazu steht SLE mit einem Defekt in Zusammenhang, der die Etablierung von B-Zell-Toleranz an diesen beiden Kontrollpunkten verhindert. Die Folge ist eine hohe Anzahl selbstreaktiver naiver B-Zellen in der Peripherie.

Das Ziel dieser Studie war die Bestimmung der molekularen Eigenschaften und Reaktivitäten von IgG Gedächtnis-B-Zell-Antikörpern in SLE. Mit Hilfe einer auf einer Einzelzell-PCR basierenden Methode wurden 200 monoklonale Antikörper von einzelnen IgG⁺ Gedächtnis-B-Zellen von vier unbehandelten SLE Patienten kloniert. Die generelle Häufigkeit an polyreaktiven und HEp-2 selbstreaktiven Antikörpern in dieser Population war vergleichbar mit der in gesunden Vergleichspersonen. 15% der Antikörper aus IgG⁺ Gedächtnis-B-Zellen eines Patienten mit Serum-Autoantikörpertitern derselben Spezifität waren hochreaktiv und spezifisch gegen die SLE-assoziierten löslichen Kernantigene Ro52 und La. Dies war jedoch nicht der Fall in den übrigen drei Patienten oder gesunden Vergleichspersonen. Die Keimbahnkonfigurationen dieser Antikörper gegen lösliches Kernantigen waren nicht-selbstreaktiv oder polyreaktiv mit schwacher Ro52-Bindung und untermauern somit die Theorie, dass somatische Mutationen zur Reaktivität und Spezifität von Autoantikörpern beitragen. Die Varianz der Häufigkeit, mit der Gedächtnis-B-Zellen SLE-assoziierte Antikörper exprimieren, legt nahe, dass dies eine wichtig Variable für den Erfolg von Therapien sein kann, die diese Population beseitigen.

SUMMARY (English)

Persistent autoantibody production in patients with systemic lupus erythematosus (SLE) suggests the existence of autoreactive humoral immunological memory, but the frequency of self-reactive memory B cells in SLE has not been determined. Under normal circumstances, autoantibodies including antinuclear antibodies (ANAs) are frequently expressed by newly generated B cells in the bone marrow, but these autoreactive B cells are tightly regulated at two checkpoints for self-tolerance, in the bone marrow and the periphery, before maturation into naïve immunocompetent B cells. In contrast, SLE is associated with a failure to establish B cell tolerance at the two checkpoints leading to high numbers of autoreactive naïve B cells in the periphery.

The aim of this study was to determine the molecular features and reactivities of IgG memory B cell antibodies expressed in SLE. A single-cell PCR based strategy was applied that allowed the cloning of the Ig heavy and Ig light chain genes of a single purified B cell and the *in vitro* expression of 200 recombinant monoclonal antibodies from single IgG⁺ memory B cells of four untreated SLE patients. The overall frequency of polyreactive and HEp-2 self-reactive antibodies in this compartment was similar to healthy controls (HC). 15% of IgG memory B cell antibodies were highly reactive and specific for SLE-associated extractable nuclear antigens (ENAs) Ro52 and La in one patient with serum autoantibody titers of the same specificity but not in the other three patients or healthy individuals. The germline forms of the ENA antibodies were non-self-reactive or polyreactive with low binding to Ro52 supporting the idea that somatic mutations contribute to autoantibody specificity and reactivity. Heterogeneity in the frequency of memory B cells expressing SLE-associated autoantibodies suggests that this variable may be important in the outcome of therapies that ablate this compartment.

Autoimmunität, SLE, Gedächtnis, Antikörper, Selbstreaktivität

Autoimmunity, SLE, memory, antibody, self-reactivity

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Abbreviations

aa	amino acid
ABTS	2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)
AID	activation-induced cytidine deaminase
ANA	antinuclear antibody
BCR	B cell receptor
CDR	complementarity determining region
CSR	class switch recombination
D	diversity
DMEM	Dulbecco's Modified Eagle Medium
DNA	deoxyribonucleic acid
ds	double stranded
DTT	dithiothreitol
ELISA	enzyme linked immunosorbent assay
ENA	extractable nuclear antigen
FACS	fluorescence activated cell sorting
FCS	fetal calf serum
FDC	follicular dendritic cell
FITC	fluorescein isothiocyanate
FWR	framework region
GC	germinal center
H	heavy
HC	healthy controls
HCMV	human cytomegalovirus

ABBREVIATIONS

HEK	human embryonic kidney
HRP	horseradish peroxidase
IFA	indirect fluorescent assay
Ig	Immunoglobulin
J	joining
L	light
LB	Lysogeny broth
LIA	line immunoblot assay
LPS	lipopolysaccharide
MHC	major histocompatibility complex
OD	optical density
PBS	phosphate buffered saline
PE	phycoerythrin
R	replacement
RAG	recombination activating gene
RBC	red blood cell
RNP	ribonuclear protein
RT	reverse transcriptase
S	silent
SHM	somatic hypermutation
SI	supplemental information
SLE	systemic lupus erythematosus
ss	single stranded
TLR	Toll-like receptor
V	variable

1 INTRODUCTION

1.1 The immune system

The immune system protects multicellular organisms from the invasion of foreign particles and the reproduction of potentially harmful microbes. In vertebrates, it is a highly interactive network, which constitutes of the innate and the adaptive branch. The prevailing property of the innate immunity is to recognize conserved structures expressed by foreign organisms, such as lipopolysaccharides (LPS) on the outer bacterial membrane, and thus acts as a rather unspecific first line of defense (Akira, et al., 2006). If this immediate response fails, the adaptive immune system is activated in the form of lymphocytes, which derive from hematopoietic stem cells in the bone marrow. Whereas T cells provide cell-mediated immunity, B cells provide humoral immunity through the secretion of antibodies. Random genetic recombination and mutational processes enable the generation of high affinity receptors with specificities against any chemical structure, but delay the onset of response. Recognition within the adaptive immune system is mediated by glycoproteins on the lymphocytes' surface, the B cell receptors (BCRs) and the T cell receptors, which belong to the immunoglobulin superfamily.

1.2 Immunoglobulins

In their basic structure, antibodies or immunoglobulins (Ig) are composed of two identical heavy (H) and light (L) chains, which are covalently connected by disulfide bonds to form a heterotetramer (Figure 1; (Davies, et al., 1975; Hill, et al., 1966)). Based on their amino acid (aa) sequences, the IgH and the two types of IgL chain, κ and λ , can be divided into a constant region towards the carboxy-terminal end and a variable region at the amino-terminal end of each chain. While the constant region determines the effector function, especially in the case of the heavy chain, the variable region shapes the antibodies specificity and affinity at the antigen binding site (Kirkham and Schroeder, 1994). The structural backbone of the variable region is provided by the four framework regions (FWR) 1, 2, 3, and 4. They are interspersed

by three segments referred to as hypervariable or complementarity determining regions (CDRs) 1, 2, and 3, which are exposed as loops at the antigen-binding region (Capra and Kehoe, 1975). CDR3 is the most variable of the CDRs and has been ascribed a dominant role in determining the antibody's specificity (Parhami-Seren and Margolies, 1996). In their membrane bound form, the IgH chains possess a hydrophobic C-terminal region spanning the cell membrane and a short intracytoplasmic tail constituting the BCR together with the invariant Ig α and Ig β chains. For the constant region of the human IgH chain five different isotypes can be distinguished based on their aa sequence. While IgM and IgD are expressed on mature lymphocytes through alternative splicing, the expression of IgG, IgA, and IgE is achieved through an irreversible genetic rearrangement process, called class switch recombination (CSR).

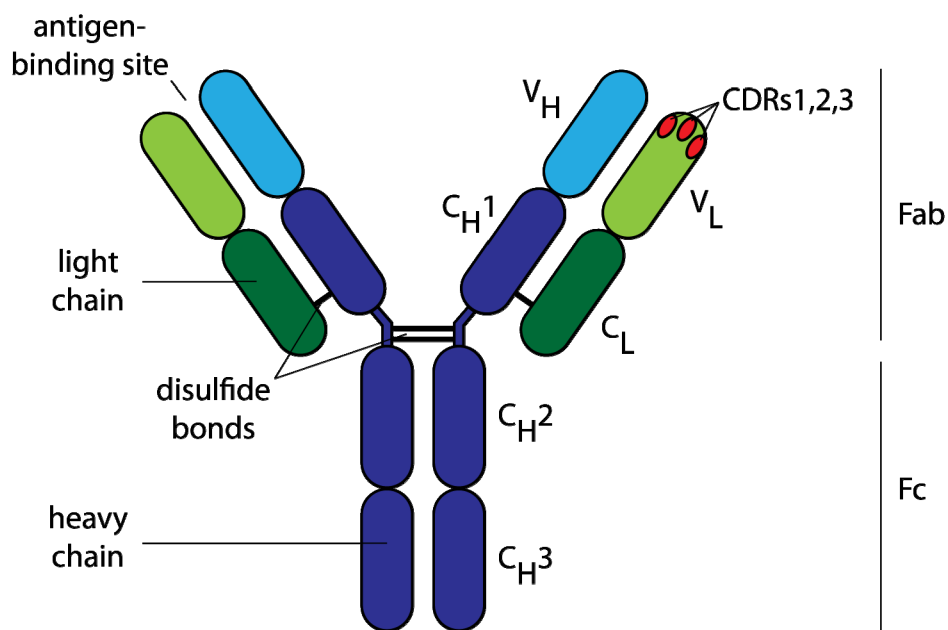


Figure 1: Schematic structure of an Immunoglobulin G. The Immunoglobulin (Ig) consists of two identical heavy (blue) and light (green) chains which are connected by disulfide bonds (black). Both heavy and light chains contain a variable (V_H and V_L, lighter color) and a constant region (C_H and C_L, darker color). The V region is folded to expose the highly variable complementary determining regions (CDRs) 1, 2, and 3 (red, visualized at V_L) at the antigen-binding site. Papain digest cleaves the Ig into the Fc and the two Fab fragments determining the Igs biological function and its specificity, respectively (modified from (Janeway, et al., 2008)).

1.3 Generation of immunoglobulin diversity

The main requisite for the generation of an antigen-specific immune response is a genetic recombination process, which takes place in the bone marrow during early lymphocyte development, called V(D)J recombination. The gene loci for the IgH, the Ig κ , and the Ig λ L chain are located on different chromosomes, with the human IgH chain locus residing on chromosome 14, and differ slightly in their organization (Croce, et al., 1979; Matsuda, et al., 1998). The variable region domain of the IgH chain is encoded by three different gene segment clusters, which are designated V (variable), D (diversity), and J (joining), followed by the constant region segments of the different Ig isotypes (Figure 2; (Brack, et al., 1978)). V, D, and J segments are not contiguous in their germline order and are recombined to generate expressable Ig genes, generally with the excision of the intervening DNA (Tonegawa, et al., 1978). Crucial for this process are two enzymes called RAG1 and 2 (from: recombination activating gene), which are expressed in developing lymphocytes and induce a double strand break of the DNA, which enables the joining of the next gene segment (McBlane, et al., 1995; Mombaerts, et al., 1992; Schatz and Baltimore, 1988; Shinkai, et al., 1992). Rearrangement on the IgH chain locus involves two steps: The recombination of a DH to a JH segment and the recombination of a VH gene segment to the DJH joint. Recombination signal sequences (RSSs), including conserved heptamer and nonamer sequences at the end of each segment, ensure that V and J of the IgH chain are not joined directly (Ramsden, et al., 1996). Imprecisions of the joining events lead to the deletion of nucleotides or the addition of untemplated N nucleotides. The DH segment can therefore be joined in any of the three reading frames and an additional diversification of the Ig gene is created (Victor and Capra, 1994). As a consequence, BCRs can differ in their specificities even when utilizing the same gene segments. The process further accounts for the extreme variability of the CDR3 which is located at the joining region of the V, D, and J segments. The gene loci of the Ig κ and Ig λ L chain of the BCR generally follow the same assembling process, though lacking D segments. Tolerance mechanism or the introduction of somatic mutations during a T-cell dependent antigen-mediated immune response can lead to further diversification of the BCR (see below).

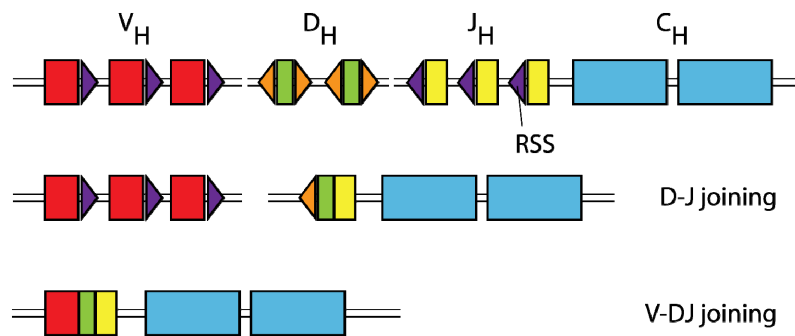


Figure 2: The V(D)J recombination. V(D)J recombination is depicted with a simplified version of the heavy chain locus. Joining of one D_H with one J_H occurs first, with the excision of the intervening DNA, guided by recombination signal sequences (RSS) (orange and purple triangles), and followed by V_H to DJH joining. The VDJ gene segment is spliced to C_μ and C_δ in pre-germinal center B cells and to either C_α, C_γ, or C_ε after class switch recombination (here: C_H, blue) (modified from (Janeway, et al., 2008)).

1.4 Immunological memory and memory B cells

Immunological memory describes the ability of the adaptive immune system to respond more rapidly and effectively to a secondary exposure with a previously encountered antigen (Steiner and Eisen, 1967). This accelerated response is provided by specifically reactive memory B and T cells, which possess intrinsic functional differences from naïve cells and thus forms the basis for vaccination. Following antigen-encounter, mature naïve B cells either directly differentiate into plasma cells (PCs) secreting low affinity antibodies or migrate into the germinal centers (GCs) to become high affinity memory B cells or plasma cells (Figure 3). GCs are specialized immunological microstructures in the spleen, tonsils, or lymph nodes, where activated B cells are brought into proximity with follicular dendritic cells and T helper cells, which present antigens and provide survival signals for B cells expressing higher affinity BCRs (Ahmed and Gray, 1996). Alteration of the BCR affinity is achieved through somatic mutations in the IgV gene segments which are randomly introduced by the RNA-editing enzyme activation-induced cytidine deaminase (AID) (Berek and Milstein, 1988). Beside the positive selection of B cells expressing high affinity receptors, called affinity maturation, CSR of the IgH chain locus to IgG, IgA, or IgE enables a more distinct effector function of the secreted antibody while retaining its specificity.

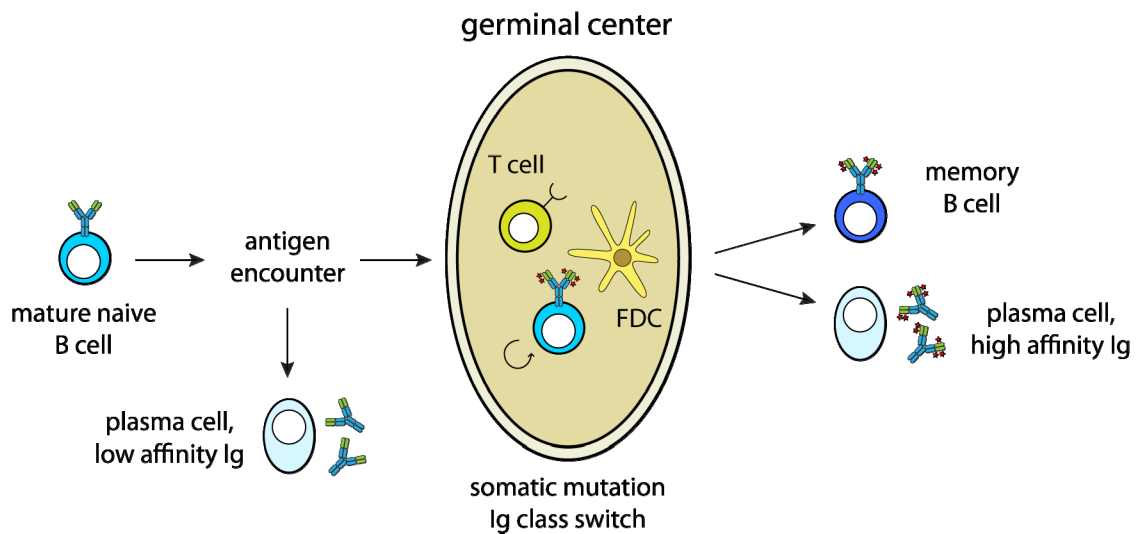


Figure 3: Scheme of memory B cell and plasma cell generation. After antigen encounter mature naïve B cells either differentiate into low affinity antibody-secreting plasma cells or proceed to germinal centers where they can acquire somatic hypermutations (red stars) and undergo class switch recombination with the help of T cells. Follicular dendritic cells (FDC) provide further antigen stimuli. B cells further mature into memory B cells or high affinity antibody-secreting plasma cells.

Memory B cell specificities correlate with antibody serum titers (Bernasconi, et al., 2002), but the direct contribution of the memory B cell pool to antibodies in the serum remains a matter of debate. Protective memory is maintained without continuous antigen-stimulation, and antigen-specific B cells and serum antibodies can be isolated more than 80 years after antigen encounter (Crotty, et al., 2003; Putz, et al., 2005; Yu, et al., 2008). One possible explanation was provided by the discovery of the long-lived PCs (Manz, et al., 1997; Radbruch, et al., 2006; Slifka, et al., 1998), yet it remained questionable whether one cell could maintain serum titers for decades, even if retained in a specialized niche, which, until now, has not been identified. Another concept focuses on the maintenance of specificities by the slow division rate of memory B cells (Macallan, et al., 2005; Wirths and Lanzavecchia, 2005). Memory B cells further express a great number of Toll-like receptors (TLRs) and can easily be activated to differentiate into antibody-secreting PCs (Bernasconi, et al., 2003; Bernasconi, et al., 2002), which could replace the long-lived PCs in their niches (Odendahl, et al., 2005). What favors the latter theory is the observation that antibody-titers of other memory specificities are elevated after a tetanus vaccine boost (Bernasconi, et al., 2002). This so called bystander activation nevertheless stresses

the possible downside of immunological memory. The beneficial effect that the prevention of reinfection has for the host, could, in the case of autoimmune disorders, lead to the persistence of autoreactivity.

1.5 Autoreactivity and tolerance

The randomness of the V(D)J recombination that forms the basis for the generation of specificities against all kinds of antigens, eventually leads to the generation of potentially pathogenic autoreactive lymphocytes (Ignatowicz, et al., 1996; Laufer, et al., 1996; Wardemann, et al., 2003; Zerrahn, et al., 1997). Paul Ehrlich proposed that the consequences of the formation of self-reactive specificities would be so severe that the immune system strictly prohibited its occurrence (Silverstein, 2001). De facto, the immune system has the ability to distinguish between self and non-self within the healthy organism. Sequential developmental tolerance checkpoints determine cell fate and prevent the maturation of highly self-reactive lymphocytes. While T cell tolerance is established in the thymus (Kisielow, et al., 1988), B cells pass a number of tolerance checkpoints along maturation. Central B cell tolerance is established in the bone marrow, where strongly autoreactive B cells are prevented from proceeding into the immature B cell stage at the first tolerance checkpoint by one or more of the following three mechanisms: deletion, anergy, and receptor editing (Gay, et al., 1993; Goodnow, et al., 1988; Nemazee and Buerki, 1989; Radic, et al., 1993; Tiegs, et al., 1993). While clonal deletion has been defined as self-antigen induced apoptosis of autoreactive B cells (Nemazee and Buerki, 1989), anergy leaves low-affinity self-reactive B cells unresponsive to further antigenic stimulation, but allows them to leave the bone marrow (Goodnow, et al., 1988; Nossal and Pike, 1980). Receptor editing enables B cells to reduce their self-reactivity by secondary recombination, predominantly at the IgL chain locus of the BCR (Gay, et al., 1993; Radic, et al., 1993; Tiegs, et al., 1993) and has been estimated to be the dominant mechanism in establishing self tolerance under physiological conditions (Casellas, et al., 2001; Halverson, et al., 2004; Retter and Nemazee, 1998). Nevertheless, this negative selection of autoreactive B cells can be bypassed. The autoantibody-encoding genes are not always inactivated in every autoreactive cell, causing allelic inclusion and the expression of BCRs with dual specificities. As a consequence, potentially pathogenic

self-reactive B cells can participate normally in immune reactions (Casellas, et al., 2007). Peripheral tolerance mechanisms in humans are less well defined but contribute to the further exclusion of self-reactive B cells in becoming fully mature (Wardemann, et al., 2003). A third checkpoint was identified on B cell progression into the IgM memory B cell compartment (Tsuiji, et al., 2006). IgG memory B cells exhibit elevated levels of self- and polyreactivity as compared to their immediate precursors, the mature naïve B cells, thus contradicting the idea of a further tolerance checkpoint. Yet, it can not be excluded that the progression of high affinity autoreactive B cells into the IgG memory B cell compartment, generated as a byproduct of the GC reaction, is prevented in healthy humans (Tiller, et al., 2007). Intermediate levels of polyreactivity have been found to occur frequently and spontaneously, even before any immune response has taken place within the organism (Ochsenbein, et al., 1999; Ochsenbein and Zinkernagel, 2000; Tiller, et al., 2007). These specificities have thus been termed natural antibodies, and it was suggested that their presence would be favorable in providing a rather unspecific but fast resistance against infections. As a consequence, it was concluded that it is more likely a matter of high frequency and reactivity than the mere presence of autoantibodies that lead to autoimmune disorders.

1.6 Autoimmunity

Defects in the establishment of tolerance enable self-reactive lymphocytes to mount immune responses against structures normally present in the host. Clinical manifestations can occur either organ-specific or systematically and often include the destruction of tissue through inflammatory processes (Davidson and Diamond, 2001). While the restriction towards certain tissues is believed to result from a limited set of autoantigens or cross-reactive specificities against exogenous antigens, systemic diseases are thought to emerge from a more global deregulation of the immune system. Systemic autoimmune diseases are mostly based on a multigenic predisposition in combination with environmental triggers, and women are generally more susceptible than men (Zandman-Goddard, et al., 2007). Alleles associated with autoimmunity effect general mechanisms such as antigen presentation, cytokine production, lymphocyte signaling, and apoptosis. Therefore, the genetic background predisposes to

more than a single autoimmune disease and increases the risk of developing multiple or overlapping manifestations. Microbial infections are regarded as an important trigger, for example, through the polyclonal activation of autoreactive cells or the generation of cross-reactive specificities that also recognize self (Bernasconi, et al., 2002; McClain, et al., 2005). Despite the overwhelming data on the presence of autoantibodies in many autoimmune diseases, their definite role in pathogenesis remains elusive. Since the exact cause of many autoimmune diseases has not been identified, therapeutic strategies focus on symptomatic treatment in addition to broadly acting immunosuppressive agents.

1.7 Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease that is characterized by a loss of self-tolerance against nuclear and cytoplasmic antigens (Kotzin, 1996). Its clinical manifestations are extremely variable and diverse and can affect the connective tissue of the whole body involving skin, joints, kidneys, and the nervous system. Disease severity has a tendency to fluctuate over time, and common symptoms include skin rashes, joint pain, and fever (Vitali, et al., 1992). SLE occurs mainly in women of childbearing age and is more frequent among people of Asian and African descent (Kotzin, 1996; Whitacre, 2001). Diagnosis is usually based on a catalog from the American College of Rheumatology consisting of 11 criteria, of which four should be fulfilled. Genetic susceptibility predisposes for disease onset, but environmental factors, such as exposure to chemicals and sunlight or viral and bacterial infections, can exacerbate the disease and trigger flares (Kotzin, 1996). A hallmark of the disease is high serum titers of anti-nuclear autoantibodies of the IgG isotype, which are present in the sera of patients long before disease onset (Arbuckle, et al., 2003). More than 100 specificities of autoantibodies have been identified (Sherer, et al., 2004), which are believed to participate directly or indirectly in SLE pathogenesis, for example through the formation of immunocomplexes and the deposition in organs, where they cause inflammation and tissue destruction (Davidson and Diamond, 2001; Koffler, et al., 1974; Kotzin, 1996; Sherer, et al., 2004). SLE has been associated with increased apoptosis and a clearance defect of apoptotic cell material (Gaipl, et al., 2007), and an early breakdown in B cell toler-

ance at the first two checkpoints in SLE leads to an increase of autoreactive mature naïve B cells in the periphery (Yurasov, et al., 2006; Yurasov, et al., 2005). Conceivable causes are a diverted cytokine milieu and defective cellular regulatory mechanisms (Lipsky, 2001). The increased presence of autoantigens and the multivalency of immunocomplexes provide a possible source for the activation of autoreactive cells and thus facilitate the generation of highly specific autoantibodies (Kotzin, 1996; Taylor, et al., 2000). Certain SLE-associated autoantibodies have further been identified to be highly reactive against exogenous antigens, such as Epstein-Barr virus surface protein (James, et al., 2001; McClain, et al., 2005). Somatic mutated anti-DNA antibodies in SLE suggest an active selection for autoantigen specificity (van Es, et al., 1991). Anti-dsDNA autoantibodies appear fairly early and titers correlate with disease activity (Hahn, 1998). Antibodies against the extractable nuclear antigens (ENAs) Ro and La play a role in skin involvement and congenital heart block in infants of serum positive mothers (Buyon, et al., 1998; Dorner, et al., 2000; Salomonsson, et al., 2005; Wahren-Herlenius and Sonesson, 2006). Standard treatment of SLE involves the administration of non-glucocorticoid immunosuppressive drugs and goes as far as B cell depletion therapy and autologous hematopoietic stem cell transplantation for severer cases.

1.8 The role of B and T cells in SLE

B cells are regarded as the main cause in SLE pathogenesis. Besides their role in the production of autoantibodies, B cells function as antigen presenters and provide immunoregulatory control of T cells, dendritic cells, and other B cells, partially through the production of cytokines (Chan, et al., 1999; Lipsky, 2001; Vallerskog, et al., 2007). The most direct evidence for the role of B cells comes from B cell ablative therapies with the chimeric human-specific anti-CD20 antibody Rituximab, which proves to be effective (Garcia-Carrasco, et al., 2009). CD20 is a B cell specific surface molecule and expressed at most stages of development. Rituximab was originally developed for the treatment of lymphomas and ablates B cells by complement-dependent cytotoxicity and antibody-mediated cell-dependent cytotoxicity (Reff, et al., 1994). The role of T cells in SLE pathogenicity is less well defined. Yet, they mediate Ig isotype class switching, somatic mutations, and affinity maturation within the proc-

ess of autoantibody production. In mice, interference in B and T cell interaction leads to disease improvement (Finck, et al., 1994; Mohan, et al., 1995). The enhanced susceptibility of carriers of particular class II major histocompatibility complex (MHC) alleles to SLE further supports the presence of autoreactive T cells in SLE (Kotzin, 1996). In addition, cytokine production of T cells contributes to the overactivation of the immune system (Morimoto, et al., 2007).

AIM

The aim of this study was to determine the frequency of self-reactivity in peripheral IgG⁺ memory B cells of SLE patients and to further characterize the molecular features of this compartment. For this, the antibodies of peripheral circulating IgG⁺ memory B cells from four untreated SLE patients were cloned, their DNA sequences analyzed, and tested in numerous reactivity assays. To determine the origin of somatically mutated SLE-specific autoantibodies cloned from the IgG⁺ memory B cells of one SLE patient, they were reverted together with a number of randomly selected antibodies from all SLE patients to their germ-line configuration and their reactivity compared to the mutated counterparts.

2 METHODS

For a detailed list of materials and equipments applied refer to supplemental materials

2.1 Production of monoclonal antibodies from single B cells

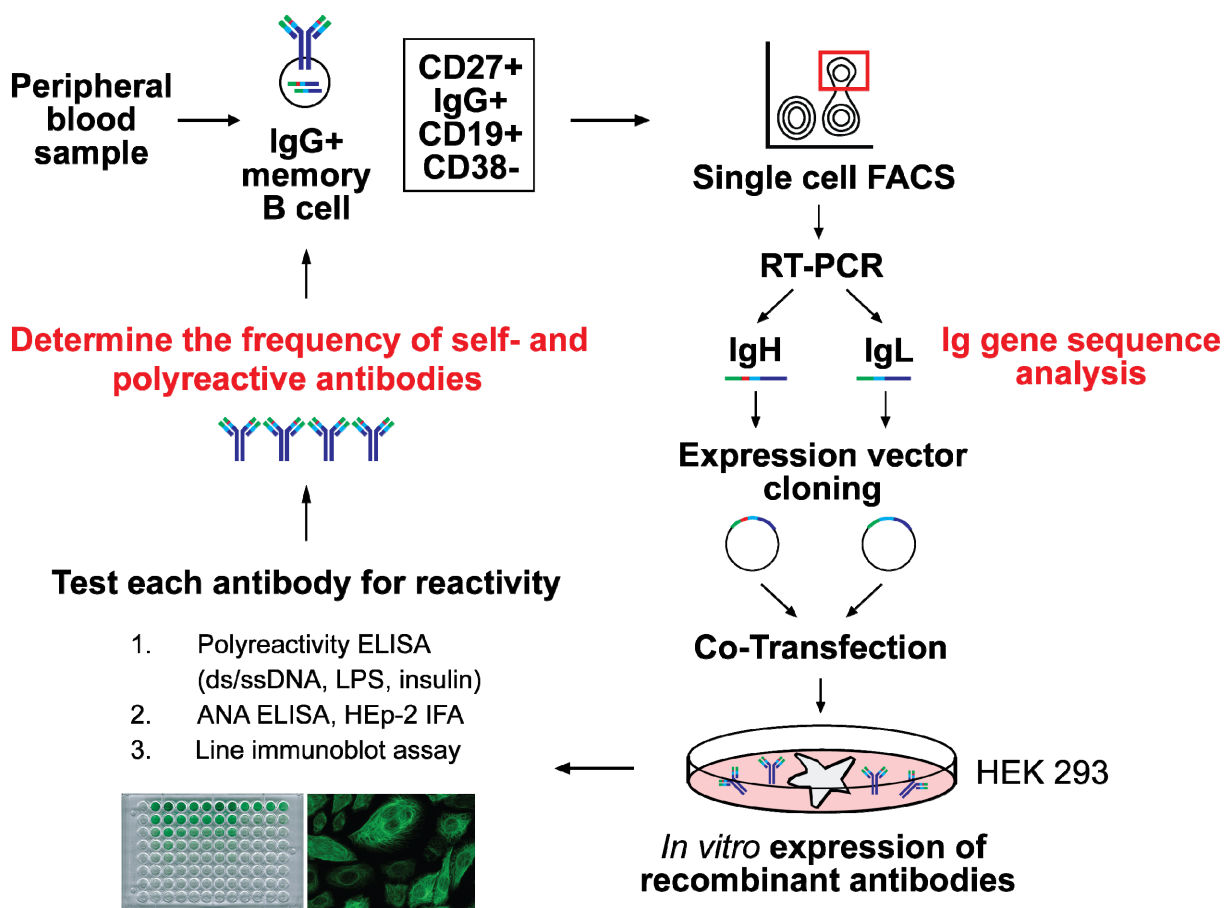


Figure 4: Strategy outline for the generation of recombinant antibodies from single B cells. IgG⁺ memory B cells from peripheral blood are stained according to surface markers and single-cell FACS sorted into 96-well plates. Following nested RT-PCR, IgH and IgL chain genes are sequenced and cloned into eukaryotic expression vectors before co-transfection into HEK 293 cells. Recombinant antibodies are harvested from supernatants and tested for reactivity in ELISA, IFA, and line immunoblot assay.

2.1.1 Isolation of single human peripheral blood B cells by fluorescence activated cell sorting

All human samples were obtained after signed informed consent in accordance with the institutional review board (IRB) reviewed protocols. Control data from IgG⁺ memory B cells of three additional healthy donors were previously published and are shown for comparison (Tiller, et al., 2008). Mononuclear cells were enriched from peripheral venous blood using RosetteSep® human B cell enrichment antibody cocktail (Stemcell Technologies Inc.) and purified by Ficoll-Paque® (GE Healthcare) density gradient centrifugation according to the manufacture's instructions. Tetrameric RosetteSep antibodies cross-link undesired mononuclear blood cells to red blood cells (RBCs) by binding to specific surface markers. After an incubation step of 20 min at ambient temperature, these immunoprecipitates pelletize together with other unbound RBCs when centrifuged over a buoyant density medium, such as Ficoll. Purified mononuclear cells are harvested from the interface.

CD19⁺CD38⁻CD27⁺IgG⁺ memory B cells and CD19⁺CD38⁺CD27⁺IgG⁺ plasmablasts were stained with anti-human antibodies CD19-allophycocyanin (APC), CD38-phycoerythrin (PE), CD27-fluorescein-isothiocyanate (FITC), IgG-Biotin, and Streptavidin-PECy7 (Invitrogen). Single cells were sorted on a FACSVantage (BD Bioscience) under discrimination of duplets into 96-well plates (Eppendorf) containing 4 µl/well ice-cold lysis buffer with 0.5x phosphate-buffered saline (PBS), 10 mM DTT (Invitrogen), 8 U RNAsin® (Promega), and 0.4 U 5 PRIME RNase Inhibitor™ (Eppendorf). Plates were sealed with Microseal® 'F' Film (BioRad), immediately frozen on dry ice, and stored at -80 °C.

2.1.2 Single cell reverse transcriptase (RT) PCR

cDNA synthesis was performed in a total volume of 14 µl/well containing 150 ng random hexamer primer (pd(N)6, GE Healthcare), 2 µl of 10 mM dNTP-Mix (Invitrogen), 1 µl 0.1 M DTT (Invitrogen), 0.5% v/v Igepal CA-630 (Sigma), 4 U RNAsin® (Promega), 6 U 5' RNase Inhibitor™ (Eppendorf), and 50 U Superscript® III reverse transcriptase (Invitrogen). RT-PCR reaction was performed according to table 1. Products were stored at -20 °C.

Table 1: RT-PCR conditions

Temperature	Time
42 °C	10 min
25 °C	10 min
50 °C	60 min
94 °C	5 min

2.1.3 IgG gene amplification

Ig γ , Ig κ , and Ig λ chain transcripts were amplified individually in two rounds of nested PCR. All reactions were carried out in 96-well plates with 40 μ l/well containing 3.5 μ l cDNA or first nested PCR product, respectively.

PCR mix: 31.7 μ l H₂O
 4.2 μ l 10x Taq buffer
 0.45 μ l dNTPs (each 25 mM)
 7 pmol Ig 5' Primer
 7 pmol Ig 3' Primer
 1.2 U HotStar® Taq polymerase
 3.5 μ l template DNA

First round of PCR was performed with a mixture of 5' L-V primers of H or κ/λ L chain as forward primers and the according reverse primer (SI: Table 12) Second round of PCR was performed with a mixture of 5' Agel VH primers and 3' IgG (internal) primer for IgH chain, 5' Pan V κ and 3' C κ 494 primer for Ig κ chain, and a mixture of 5' Agel V λ primers and 3' XhoI C λ for Ig λ . Each round of PCR, consisting of 50 cycles, was performed according to table 2. PCR products were stored at -20 °C.

Table 2: IgG gene PCR conditions

Temperature	Time
94 °C	30 s
58 °C (γ, κ)/60 °C (λ)	30 s
72 °C	55 s (1 st PCR)/45 s (2 nd PCR)
4 °C	hold

2.1.4 Ig gene sequence analysis

Products of the second PCR were sequenced with the respective reverse primers (SI: Table 12) and analyzed for Ig gene usage, CDR3 features, and number of V gene mutations using IgBLAST (<http://www.ncbi.nlm.nih.gov/igblast/>). CDR3 length was determined by counting the aa following FWR3 up to the conserved tryptophan-glycine motif in JH or the phenylalanine-glycine motif in J κ and J λ . The number of positively (arginine (R), histidine (H), lysine (K)) and negatively (aspartate (D), glutamate (E)) charged aa residues were identified for each IgH and IgL CDR3. IgH chain D gene reading frames were determined according to Corbett et al. (Corbett, et al., 1997). For the analysis of replacement (R) and silent (S) mutations each nucleotide was considered independently unless in rare cases where a combination of nucleotide exchanges within one codon was needed to replace or maintain a specific aa. R and S frequencies were calculated according to the number of nucleotides in the respective V gene as defined by IgBLAST. Polymorphisms were identified by comparison to published germline sequences (<http://www.ncbi.nlm.nih.gov/igblast/>) or own observations.

2.1.5 Cloning of PCR amplifications into expression vectors

To enable cloning of IgH and Ig κ products lacking restriction sites, second PCR was repeated as a specific PCR using the respective 5' AgeI VH and 5' AgeI V κ gene-specific forward primers and 3' SalI JH and 3' BsiWI J κ reverse primers according to gene information received from sequence analysis. The PCR products of the

specific PCR for IgH and Ig κ and 2nd PCR for Ig λ were purified using Qiaquick® 96 PCR Purification Kit (Qiagen) and QIAvac 96 according to the manufacturer's instructions. Samples were eluted with 50 μ l nuclease-free water (Eppendorf) resulting in a volume of 30-35 μ l purified DNA. Digests were undertaken with restriction enzymes AgeI, SalI, BsiWI and XhoI (all New England BioLabs) in the according buffer at 37 °C (except BsiWI at 55 °C) for at least 1h. Following a further purification step, as described above, ligation was carried out in a total volume of 10 μ l with 1 U T4 DNA-Ligase (Invitrogen), 7 μ l digested and purified PCR product and approximately 25 ng of the respective linearized human Ig γ 1, Ig κ , and Ig λ expression vector. The vectors contained a multiple-cloning site and an ampicillin resistance gene and transcription was under the control of the human cytomegalovirus (HCMV) promoter.

2.1.6 Amplification of vector DNA

For amplification of vector DNA, 10 ng vector DNA was transformed into competent *E. coli* as stated below (see 2.1.9). A single colony was picked from the Lysogeny broth (LB) agar plate and grown in 2.5 ml LB with 75 μ g/ml ampicillin at 37 °C in the incubator for 16 h, before transferring the suspension to 250 ml LB with 75 μ g/ml ampicillin for another 16 h in the incubator. Vectors were obtained from the bacterial culture by basic lysis and purified using HiSpeed® Plasmid Maxi Kit (Qiagen) following the manufacturer's instructions. Linearization was performed with the respective enzymes (SI: Figure 30). Products were separated on a 1% agarose gel at 100 V for 30 min, cut from the gel, and purified with a Qiaquick® Gel Extraction Kit (Qiagen), according to the protocol.

2.1.7 Production of competent bacteria

Competent bacteria were produced via calcium chloride treatment. For this, bacteria of the *E. coli* DH10B strain (Clontech) were plated on LB agar plates, and a single colony was grown in 4 ml LB medium at 37 °C and 170 rpm for 16-18 h. The suspension was transferred into a 1 l Erlenmeyer-flask together with 500 ml LB medium, grown at 37 °C and 170 rpm until an OD of 0.5-0.8 at 600 nm, equally divided onto four 400 ml centrifugation tubes, and incubated on ice for 30 min. After centrifu-

gation at 1600 xg for 10 min at 4 °C, the pellet was resuspended in 80 ml 0.1 M CaCl₂ and kept on ice for a minimum of 2 h before an additional round of centrifugation (as above). The supernatant was discarded, the pellet resuspended in 2 ml 0.1 M CaCl₂ with 15% v/v glycerol, immediately frozen in 100 µl aliquots on dry ice, and kept at -80 °C until further use. Competence was confirmed and determined by counting the colonies after transformation of different amounts of vector DNA ranging between 0.3 ng to 30 ng DNA. Transformation was performed as described below. Numbers of colonies were divided by the amount of DNA (µg) and multiplied with the ratio of the recovered volume of bacteria (ml) and the volume plated (ml). Typical values obtained ranged between 5 x 10⁵ to 10⁶ colonies per µg DNA.

2.1.8 Reversion strategy of mutated IgH and IgL chain genes to germline form

An overlap-PCR strategy was used to revert mutated V(D)J gene segments of the IgH and IgL chain to their germline form (Figure 5). V gene segments were amplified from unmutated templates previously cloned with a gene-specific forward primer containing the AgeI restriction site and an individual reverse primer annealing to the 3' end of the FWR3 region. CDR3-J segments were reverted with an individual primer containing a minimal complementarity of 10 nucleotides with the reverse V segment primer. J segment reverse primers contained the respective restriction site. PCR was performed at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 45 s (see also table 2, according to conditions for γ, κ and 2nd PCR) for 30 cycles. Equal amounts of PCR products were fused in a 20-cycle overlap PCR under the same conditions. Bands of the correct full-length size as compared to the respective mutated genes were cut and eluted from the gel before restriction and cloning into the respective vectors. Gene identity, successful reversion, and correct insertion into the vector were confirmed by sequencing the product of the bacterial colony PCR after transformation into *E. coli* DH10B, as described below.

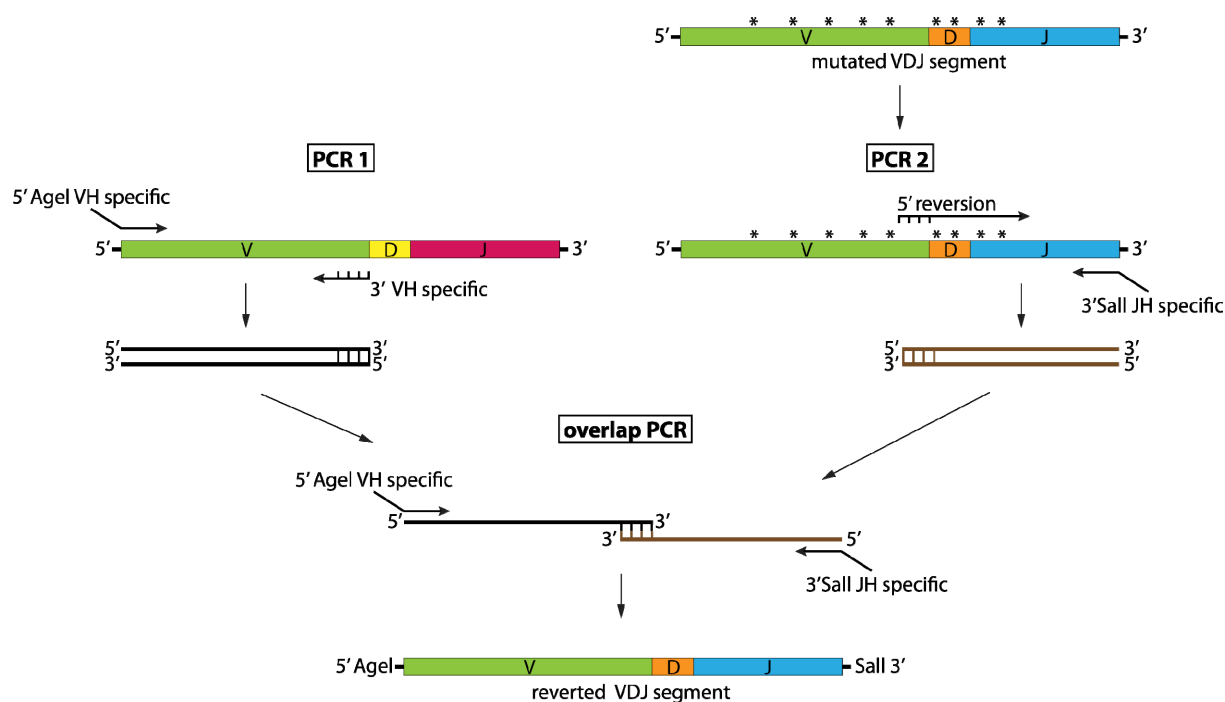


Figure 5: PCR strategy for the reversion of somatically mutated Ig genes to their germline counterparts. Reversion is exemplified on an IgH chain. Asterisks indicate somatic mutations. PCR 1 amplifies a germline VH segment corresponding to the mutated VH segment. Primers in PCR 2 revert mutations in CDR3 region and JH segment of the mutated clone. Homology of 3' VH specific primer (PCR 1) and 5' reversion primer (PCR 2) are indicated by vertical lines. Products from both PCRs are fused in a subsequent overlap PCR using the forward primer of PCR 1 and the reverse primer of PCR 2 to generate the complete germline VDJ segment. (modified from (Tiller, et al., 2008))

2.1.9 Transformation of vector DNA into competent *E.coli*

Competent bacteria *E. coli* DH10B were transformed via heat shock at 42 °C for 50 sec with 3 µl ligation product. In detail, 7 µl bacteria were mixed with the DNA in a 96 well plate (Costar) and incubated on ice 30 min before and 5 min following heat shock. 100 µl LB without antibiotics was added to each well and bacteria were grown for 30 min at 37 °C in the incubator before plating them on 10 cm LB agar plates with 75 µg/ml ampicillin (Sigma). Following an over night incubation at 37 °C in the incubator, colonies were screened by PCR with 5' Ab sense as a forward primer and 3' IgG (internal), 3' C κ 494, and 3' C λ as reverse primers, respectively. PCR products were sequenced to confirm correct insertion into the expression vector according to reading frame. Sequences were further blasted using SeqManII (DNASTAR) against the original PCR product sequence to exclude mutations intro-

duced by error-prone Taq polymerase. Plasmid DNA was obtained from 3.5 ml 16-18 h cultures of bacteria in Terrific Broth (Carl Roth) with 75 µg/ml ampicillin (Sigma) by basic lysis using QIAprep® Spin columns (Qiagen).

2.1.10 Production of recombinant antibodies

Human embryonic kidney (HEK) 293T (ATCC, No. CRL-11268) cells were cultured in 150 mm plates (Falcon, Becton Dickinson) under standard conditions (37 °C in a humidified atmosphere containing 5% CO₂) in Dulbecco's Modified Eagle's Medium (DMEM; GibcoBRL) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Invitrogen), 1 mM sodium pyruvate, 100 µg/ml streptomycin, 100 U/ml penicillin G, and 0.25 µg amphotericin B (all GibcoBRL). Heat-inactivation of FCS was performed for 30 min at 56 °C in a water bath before refreezing 50 ml aliquots and storing them at -20 °C until further use.

Cells were expanded every two days at a confluency of 80% at a 1:10 dilution. For this, cells were washed with 10 ml PBS and incubated in 3 ml trypsin (GibcoBRL) for 30-60 s. After trypsin removal and loosening of the cells by repeated tapping of the plate, cells were suspended in 20 ml growth medium and 2 ml cell suspension were transferred onto a new plate with 20 ml medium.

Transient transfection with an IgH chain vector and its respective IgL chain vector was performed by calcium-phosphate precipitation. In detail, expression vector DNA was mixed with 0.7 mM Chloroquine (Sigma) in 1 ml sterile water. 2.5 M CaCl₂ was added drop-wise to a concentration of 250 mM. An equal volume of 2x HEPES-buffered saline (50 mM HEPES, 10 mM KCl, 12 mM Dextrose, 280 mM NaCl, 1.5 mM Na₂HPO₄·7H₂O, pH 7.05) was slowly added continuously vortexing the solution before incubating the whole at room temperature for 10 min to allow the formation of precipitates. The precipitation-mixture was distributed equally over HEK cells with a confluency of 80%. After 8-12 h in the incubator, HEK cells were washed twice with 10 ml serum-free DMEM and kept in 25 ml DMEM supplemented with 1% Nutridoma-SP (Roche) and antibiotics as stated above for 6 d. Supernatants were harvested and cell debris removed by centrifugation at 800 xg for 10 min at 4 °C before storage at 4 °C with 0.05 % sodium azide. Production of recombinant antibodies was confirmed by enzyme-linked immunosorbent assay (ELISA).

2.1.11 Purification of recombinant antibodies

Recombinant antibodies were purified from the supernatants according to the manufacturer's protocol using Protein G beads (GE Healthcare). Protein G is a streptococcal cell-surface protein with three homologous binding sites that bind IgG with high affinity. Briefly, 25 ml supernatant were incubated with 25 μ l washed Protein G beads on a rotating shaker at 4 °C overnight. After centrifugation at 800 xg for 10 min at 4 °C the supernatant was removed and the beads transferred to a chromatography spin column (BioRad) equilibrated with PBS. After two rounds of washing with 1 ml PBS, bound recombinant antibodies were removed from the beads in three elution steps with 200 μ l 0.1 M glycine (pH 3.0) each and collected in tubes containing 20 μ l 1 M Tris (pH 8.0) with 0.5 % sodium azide.

2.1.12 IgG concentration ELISA

High-binding capacity ELISA plates (Costar) were coated with 50 μ l/well goat anti-human IgG (Fc γ -specific; Jackson) at a concentration of 2 μ g/ml in PBS overnight. Antibody concentrations of HEK cell supernatants were determined using a 3 μ g/ml human serum IgG1 (Sigma) diluted in PBS as a standard. Purified antibodies were diluted between 1:50 to 1:150 according to the estimated relative concentration by determination of the optical density (OD) at 280 nm. ELISA plates were washed three times with 200 μ l/well water, blocked with 200 μ l/well blocking buffer (2 mM EDTA and 0.05 % Tween®-20 in PBS) for 1 h and washed again. Antibodies were incubated for 2 h. After a further washing step ELISA plates were incubated with 50 μ l/well horseradish-peroxidase (HRP) conjugated goat anti-human IgG (Jackson) at a concentration of 0.8 μ g/ml in blocking buffer for 2 h. Unbound antibody was removed by two washing steps including an intermediate rinsing step with 200 μ l/well blocking buffer. Assays were developed with HRP chromogenic substrate 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS; Pierce) according to the manufacturer's instructions, and OD was measured at 405 nm using SoftMax Pro® software (Molecular Devices). All steps were performed at ambient temperature.

2.2 Assays determining antibody reactivity

2.2.1 Polyreactivity ELISA

Incubation, blocking, and washing was performed as described above for IgG concentration ELISA. Antibody supernatants were tested at a concentration of 1 µg/ml with three consecutive 1:4 dilutions in PBS. High-binding capacity ELISA plates (Costar) were coated with 50 µl/well of one of the following antigens: Insulin (5 µg/ml), double-stranded (ds) DNA, single-stranded (ss) DNA, or lipopolysaccharide (LPS) (all 10 µg/ml). ssDNA was prepared from salmon sperm dsDNA by boiling at 94 °C for 30 min and immediately freezing aliquots at -20 °C. Human recombinant insulin solution (Sigma) and LPS from *E. coli* Serotype 055:B5 (Sigma) were stored at 4 °C. For the detection of bound antibodies ABTS (BioRad) was used as a substrate. Controls for polyreactivity were the human recombinant antibodies mGO53 (negative; (Wardemann, et al., 2003)), eiJB40 (low positive; (Wardemann, et al., 2003)) and ED38 (high; (Meffre, et al., 2004)) and were included in each plate. Cut-off OD above which an antibody was counted as reactive was determined for each experiment by taking the average OD of eiJB40 and subtracting two times the standard deviation at a concentration of 1 µg/ml. A minimum of three controls was included in each experiment to allow calculation of standard deviation. An antibody was counted as polyreactive if it bound at least two structurally different antigens and if reactivity was confirmed in at least two independent experiments.

2.2.2 Anti-nuclear antibody (ANA) ELISA

Self-reactivity of protein G-purified monoclonal recombinant antibodies was tested using QUANTA Lite™ ANA ELISA plates coated with HEp-2 cell lysates enriched for nuclear antigens (INOVA Diagnostics). Assay procedures were identical to polyreactivity ELISA, but starting with an antibody concentration of 10 µg/ml in PBS. Negative and low positive controls were used as provided by the manufacturer and contained sera from healthy individuals and patients, respectively. Antibodies in ANA ELISA were considered self-reactive if reactivity was confirmed in HEp-2 immunofluorescence or immunoblot assay or if they showed strong HEp-2 immunofluo-

rescence staining patterns (usually against cytoskeletal antigens which were not abundant in the ANA ELISA).

2.2.3 Anti-Ro/SSA and Anti-La/SSB ELISA

Anti-Ro/SSA and anti-La/SSB reactive protein G-purified monoclonal recombinant antibodies in their mutated and reverted form were tested on ELISA plates containing purified recombinant Ro/SSA or La/SSB protein (ORGENTEC Diagnostika). Assay procedures were identical to polyreactivity ELISA, but starting with an antibody concentration of 1 µg/ml in PBS for Ro/SSA and 10 µg/ml in PBS for La/SSB due to less intense reactivity of anti-La/SSB antibodies in ANA ELISA.

2.2.4 HEp-2 immunofluorescence assay

HEp-2 cell coated slides (BIOS GmbH) were incubated in a moist chamber with 20 µl purified antibodies at a concentration of 50-150 µg/ml for 30 min. Slides were washed three times in PBS for 5 min and subsequently incubated with 20 µl FITC-labeled goat anti-human Ig according to the manufacturer's instructions. After an additional washing step, slides were coated with Fluoromount-G (SouthernBiotech) and sealed with a glass cover slip. Edges between slide and cover slip were additionally sealed with nail polish. Control stainings, including PBS, ANA-negative, and positive control, were applied as suggested by the manufacturer and incorporated in all assays. Samples were examined on a Zeiss Axioplan 2 fluorescence microscope (Carl Zeiss MicroImaging). Stainings were evaluated in comparison to controls at equal exposure times.

2.2.5 Immunoblot assay

Immunoblot assay (INNO-LIA ANA Update; Innogenetics) was performed to determine reactivity of purified recombinant antibodies with specific disease-related antigens according to the manufacturer's instructions. In brief, antibodies were tested at a concentration of 2 µg/ml or human serum at a dilution of 1:100 in the provided solution for 1 h. After three times washing for 3 min, the blot stripes were incubated in

2 ml of the conjugate-solution, containing an alkaline phosphatase-labeled goat anti-human IgG antibody, for 1 h. After an additional washing step, bound antibody was made visible by adding 2 ml of the chromogen BCIP/NBT. After 30 min, further staining was prevented by transferring the stripes into sulfuric acid to inhibit the activity of the alkaline phosphatase. A cut-off control was included in each assay.

2.3 Statistics

2.3.1 Statistical analysis

P-values for Ig gene repertoire analysis, analysis of positive charges in IgH CDR3, and antibody reactivity were calculated by 2 x 2 or 2 x 5 Fisher's Exact test or Chi Square Test. P-values for IgH CDR3 length were calculated by non-paired two-tailed Student's *t*-test. P-values for V gene FWR1-FWR3 mutations were calculated by non-parametric Mann-Whitney U test. P-values for ratios of VH to VL gene SHM were calculated by 1 way ANOVA analysis.

3 RESULTS

3.1 Clinical features of four analyzed SLE patients

The blood samples were obtained from four newly diagnosed, untreated, pediatric SLE patients (169, 174, 175, and 176) with active disease. The patients' clinical features were diverse as were the autoantibody specificities in the patients' sera reflecting the heterogeneity of the disease. All of the patients were anti-nuclear antibody (ANA) positive with different serum specificities including reactivities against dsDNA, Smith-antigen, Cardiolipin, ribonuclearproteins (RNP), and other ENAs. Two patients showed lupus nephritis and three patients exhibited a decrease in complement components (Table 3).

Table 3: Patients' characteristics

Patient	SLE169	SLE174	SLE175	SLE176
Gender	F	F	F	F
Age (yr)	12	17	11	13
Treatment	none	none	none	none
Clinical features	arthritis	malar rash, arthritis	pain, hypothyroidism	rash
Lupus nephritis	IV	/	/	V
ANA	+	+	+	+
C3 complement (mg/dl)	28	78	176	65
C4 complement (mg/dl)	<6	15	40	4
Serology	D, CL	ENA/RNP, Sm	ENA/RNP, Sm	D, ENA/RNP, Sm

D, anti-dsDNA antibodies; CL, anti-cardiolipin antibodies; Sm, anti-Smith antibodies; ENA/RNP, anti-extractable nuclear antigen/anti-ribonucleoprotein antibodies; normal values for complement: C3 90-180 mg/dl, C4 10-40 mg/dl (Herold, 2003).

3.2 B cell FACS staining patterns of SLE patients

To characterize the IgG antibodies expressed by circulating memory B cells in SLE, IgG⁺ memory B cells (CD19⁺CD27⁺IgG⁺CD38⁻) were isolated from peripheral blood of the patients by FACS (Figure 6). A previously reported increased population of CD38⁺CD27⁺⁺ plasmablasts with low levels of IgG was only found in patient SLE169 (Figure 6; (Arce, et al., 2001; Odendahl, et al., 2000; Vallerskog, et al., 2007)). The IgH and IgL chains were cloned from the IgG⁺ memory B cells as previously described (chapter 2.1 and (Tiller, et al., 2008)). None of our reactivity assays showed a significant difference between CD38⁻ memory B cells and CD38⁺ plasmablasts in SLE169, and therefore these were considered together (Figure 6 and SI: Table 5).

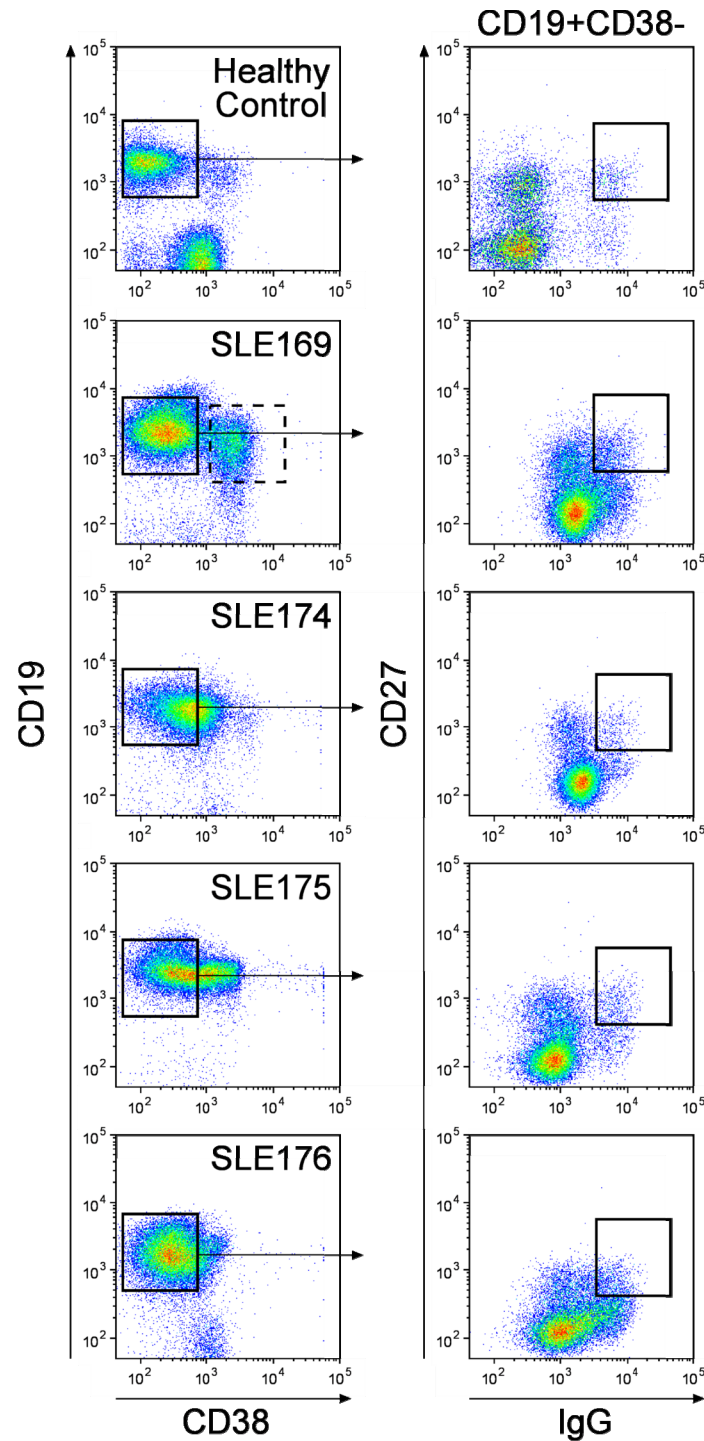


Figure 6: Surface staining and gates for FACS purification of single IgG+ memory B cells from healthy controls and SLE patients. Pre-enriched B cells were stained for surface expression of CD19, CD38, CD27 and IgG. CD19⁺CD38⁻ cells and CD19⁺CD38⁺ cells (left) were further analyzed for CD19 and IgG expression (right). Boxed CD38⁻CD19⁺IgG⁺CD27⁺ memory B cells from SLE169, SLE174, SLE175, and SLE176 and CD38⁺CD19⁺IgG⁺CD27⁺ plasmablasts from SLE169 were single cell FACS sorted (right) before amplification of Igγ H and corresponding IgL chains (SI: Tales 5-9).

3.3 Molecular features of SLE IgG⁺ memory B cells

3.3.1 IgH chain gene usage and CDR3 characteristics

None of the analyzed sequences were clonally related (SI: Table 5-9) and the majority of the functional genes were used in SLE IgG⁺ memory B cells (SI: Table 5-8; (Lefranc, et al., 2005)). Previous reports showed abnormalities in the distribution of IgVH chain gene segments in different developmental B cell stages of SLE patients as compared to HC (Dorner, et al., 1999; Fraser, et al., 2003; Pugh-Bernard, et al., 2001). The comparison of the VH gene family usage of IgG⁺ memory B cells of SLE patients and HC showed no significant difference. In both groups VH3 was predominantly expressed, followed by VH4, VH1, VH5, and VH7 family members (Figure 7A top and SI: Figure 21A). IgH chain diversity (D) and joining (J) gene segments were also comparable in their distribution among SLE patients and HC, with the majority of the B cells expressing DH3 and nearly half expressing JH4 (Figure 7A middle and bottom and SI: Figure 21A).

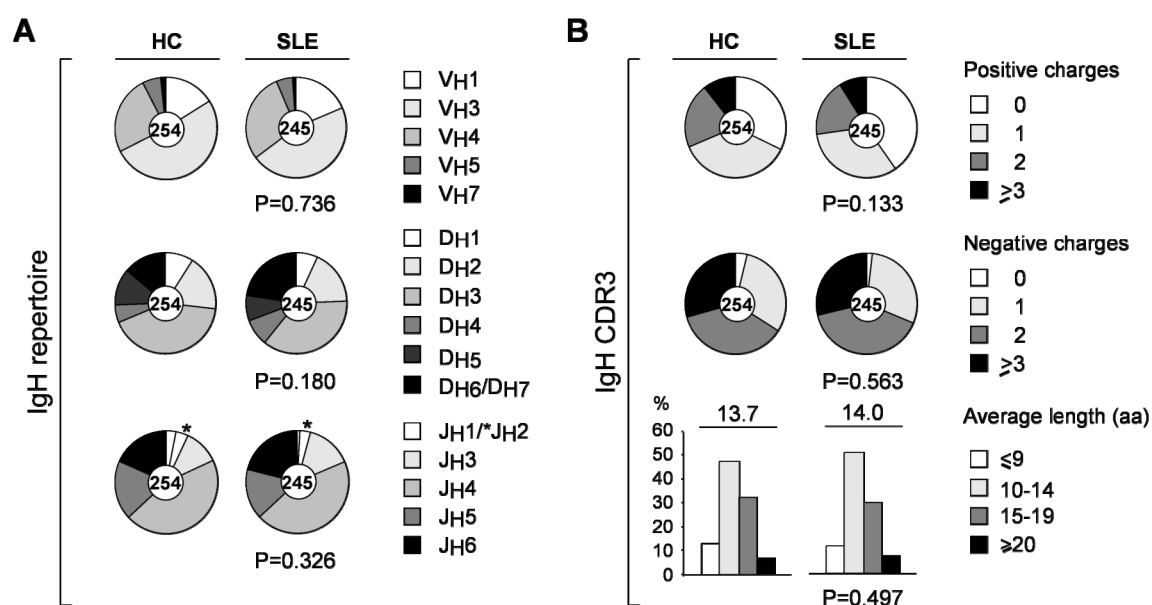


Figure 7: IgH chain gene features from IgG memory B cell antibodies of SLE patients and healthy controls. Ig gene repertoire and Ig gene features of IgG memory B cell antibodies cloned from three published (Tiller, et al., 2007) and one unpublished healthy control (HC-JH) and four patients with SLE (SLE169, SLE174, SLE175, SLE176). P-values compare data from the patients to the healthy controls. Pie charts depict VH, DH and JH gene usage (A) and the frequency of IgH CDR3s with 0, 1, 2 or ≥ 3 positive (top) and negative (middle) charges (B). The absolute number of sequences analyzed is indicated in the center of each pie chart. Bar graphs show the frequency of antibodies with CDR3 regions of ≤ 9 aa (white bars), 10-14 aa (light gray bars), 15-19 aa (dark gray bars) or ≥ 20 aa (black bars). The average CDR3 length in aa is indicated above the bar graphs.

CDR3 features such as charged aa and length have been associated with autoreactivity (Crouzier, et al., 1995; Radic, et al., 1993). To determine if abnormalities in the CDR3s of IgG⁺ memory B cells are a feature of SLE, the number of positively (arginine, lysine, histidine) and negatively (aspartic acid, glutamic acid) charged aa were quantified as a first step. CDR3s lacking positively charged aa were increased in SLE as compared to HC, but this did not reach statistical significance (40.0% in SLE vs. 32.3% in HC, $P=0.133$; Figure 7B top). The frequency and distribution of negatively charged aa was nearly identical in SLE and HC ($P=0.563$; Figure 7B middle). The analysis of the CDR3 length as determined by the number of aa showed no significant difference between SLE and HC (14.0 vs 13.7, $P=0.497$; Figure 7B bottom). This held true if either the average CDR3 lengths were compared or the distribution of CDR3 length ranges among different clusters. About half of the CDR3 lengths belonged to the cluster containing 10-14 aa (50.6% in SLE and 47.5% in HC), and extremely long CDR3s with 20 aa and more were found in less than 8% of the antibodies in both SLE and HC (7.8% in SLE and 7.1% in HC; Figure 7B bottom).

3.3.2 IgL chain gene usage

Differences in the usage of IgL chain genes have been reported to be a feature of SLE (de Wildt, et al., 2000; Dorner, et al., 1999). Therefore, the usage of Ig κ and Ig λ L chain gene segments between IgG⁺ memory B cells of SLE patients and HC was compared. Ig κ L chain usage in SLE differed significantly from normal through an increase in V κ 2 family usage (13.8% in SLE vs. 4.7% in HC, $P=0.027$; Figure 8A and SI: Figure 22A). The expression of individual V κ gene segments was further analyzed and revealed an underrepresentation of V κ 3-20, which was consis-

tent in all SLE patients (on average 10.3% in SLE and 26.2% in HC; SI: Figure 25 and 26). The J_{κ} usage was comparable between SLE and HC with $J_{\kappa}1$ being primarily expressed followed by $J_{\kappa}2$ and $J_{\kappa}4$. Significant differences in $Ig\lambda$ L chain usage could not be observed. $V\lambda 1$ and $V\lambda 2$ were primarily expressed in both groups (36.5% and 40.5% in SLE vs. 41.0% and 38.6% in HC, respectively). $V\lambda 3$ was enhanced in SLE (16.7% in SLE vs. 8.4% in HC) and $V\lambda 7$ was reduced (0.8% in SLE vs. 8.4% in HC), but this did not reach statistical relevance ($P=0.072$; Figure 8B top). Further did the inverse distribution of $J\lambda 2$ and $J\lambda 3$ between SLE and HC not lead to a significant difference (35.7% and 46.8% in SLE and 44.6% and 37.3% in HC, respectively, $P=0.430$; Figure 8B bottom). However, SLE patients exhibited a consistent overexpression of $Ig\lambda$ L chain genes (46% in SLE vs. 32% in HC, $P=0.0004$; Figure 8 and SI: Table 5-8; (Tiller, et al., 2007)).

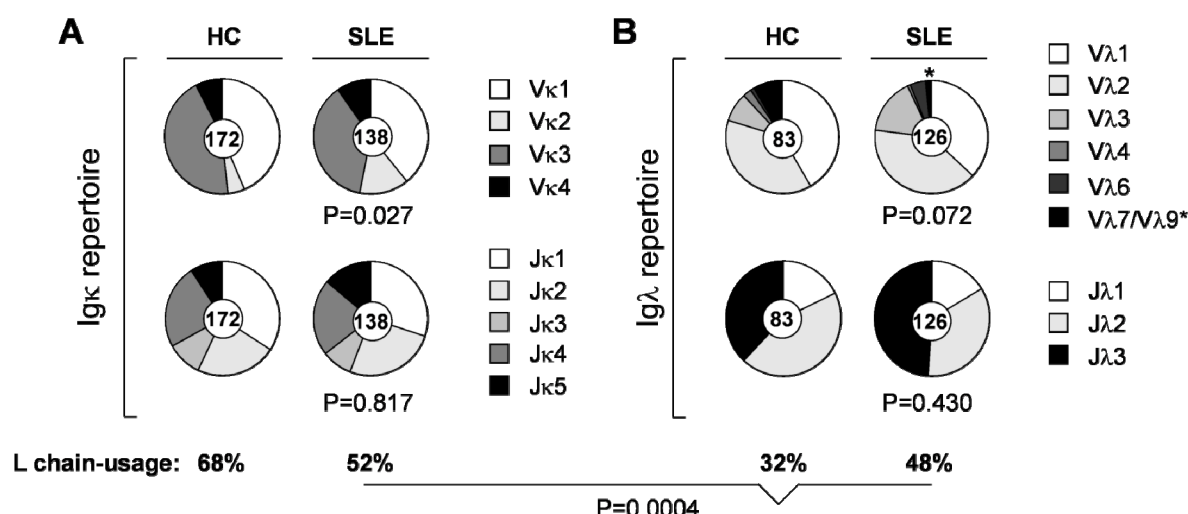


Figure 8: IgL chain gene features from IgG memory B cell antibodies of SLE patients and healthy controls. Ig gene repertoire and Ig gene features of IgG memory B cell antibodies cloned from three published (Tiller, et al., 2007) and one unpublished healthy control (HC-JH) and four patients with SLE (SLE169, SLE174, SLE175, SLE176). P-values compare data from the patients to the healthy controls. Pie charts depict V_{κ}/J_{κ} (A) and $V\lambda/J\lambda$ gene family usage (B). The absolute number of sequences analyzed is indicated in the center of each pie chart. The frequency of $Ig\kappa$ and $Ig\lambda$ light chain positive antibodies is indicated below.

3.3.3 Individual differences of SLE patients

Analyzing the SLE patients individually revealed a number of unusual features. SLE174 showed a significant difference in the DH repertoire. DH1 and DH3

were reduced (2.2% and 32.6% in SLE vs. 8.9% and 41.9% in HC, respectively) while DH4 and DH6 were increased (15.2% and 26.1% in SLE vs. 5.6% and 14.0% in HC, respectively) ($P=0.047$; SI: Figure 21A middle). Even more striking were the individual differences in SLE169. First of all, the CDR3 length showed a significant increase (average 15.1 aa vs. 13.9 aa in HC, $P=0.012$; SI: Figure 21B bottom). Analyzing the single VH gene segments showed an overrepresentation of VH 4-4 (15.6% vs. 1.6% in HC and 2.2% in the other SLE patients; SI: Figure 23 and 24 and Tables 5-9 (Tiller, et al., 2007)). The IgV L chain repertoire exhibited a lower frequency of the V λ 2 gene family (25.0% vs. 38.6% in HC), whereas the V λ 3 family gene usage was increased in SLE169 (25.0% vs. 8.4% in HC, $P=0.005$; SI: Figure 22B top). IgG⁺ memory B cells from this patient were further unusual in that >25% of the B cells expressed both a functional Ig κ and Ig λ L chain as compared to 0% in healthy controls and 0-2% in the other SLE patients (SI: Table 5 and 10 (Tiller, et al., 2007)).

To elucidate possible mechanisms behind allelic inclusion such as tolerance induction in Ig κ^+ Ig λ^+ double expressing B cells in SLE169, self-reactivity of the antibodies with either Ig κ or Ig λ L chain were compared. Ig λ L chain antibodies exhibited autoreactivity only in concordance with the respective Ig κ L chain antibody, whereas one third of the antibodies were self-reactive only with the Ig κ L chain (Table 4).

Table 4: Self-reactivity of antibodies with two functional IgL chains from patient SLE169

$\kappa+\lambda+$	λ		
	SLE169	self-reactive	non self-reactive
κ	self-reactive	13.3%	33.3%
	non self-reactive	0%	53.3%

3.3.4 Secondary rearrangement and IgV to IgJ gene segment joining in SLE

Secondary rearrangement on the IgL chain loci as a mechanism to alter receptor specificities has been evident in transgenic mice expressing an anti-dsDNA-reactive H chain, through an increased usage of the most downstream J κ 5 gene segment (Radic, et al., 1993). In human autoimmune diseases, an increase as well as a decrease of secondary L chain rearrangement has been reported (Bensimon, et al., 1994; Dorner, et al., 1998; Samuels, et al., 2005; Yurasov, et al., 2006). Due to their spatial proximity on the gene locus, VL chain gene segments are most likely joined to upstream JL chain gene segments in a primary rearrangement. During further rearrangement they are deleted from the locus so that an increased joining of upstream V gene segments to downstream J gene segments argues for enhanced secondary rearrangement. A distinct feature of the Ig κ locus, however, is that a number of V gene segments are present in the reverse orientation to that of the J gene segments. They rearrange by inversion, thus retaining the V gene segments further downstream, which can then be used during a further rearrangement (Schroeder, 2006). A primary rearrangement can therefore only be identified through the usage of J κ 1.

To check for major changes in the joining of V κ to J κ gene segments in SLE IgG⁺ memory B cells, the events within a single cell were visualized in a graph with the V gene segments on the ordinate and the J gene segments on the abscissa (Figure 9). The increased usage of J κ 1 or J κ 2 would argue for an increased presence of primary rearrangements. A cluster of joining events in the upper right corner would argue for an increase in secondary rearrangement. A major difference in the distribution of V κ to J κ joining events between SLE and HC was not observed (Figure 9). The majority of the events clustered around the individual genes mostly used, e.g. V κ 3-20 and V κ 1-5 (compare also SI: Figures 25 and 26), and the frequency of J κ 3, 4, and 5 joining events to V κ 3-15 and above was comparable (27% for HC and 23% for SLE).

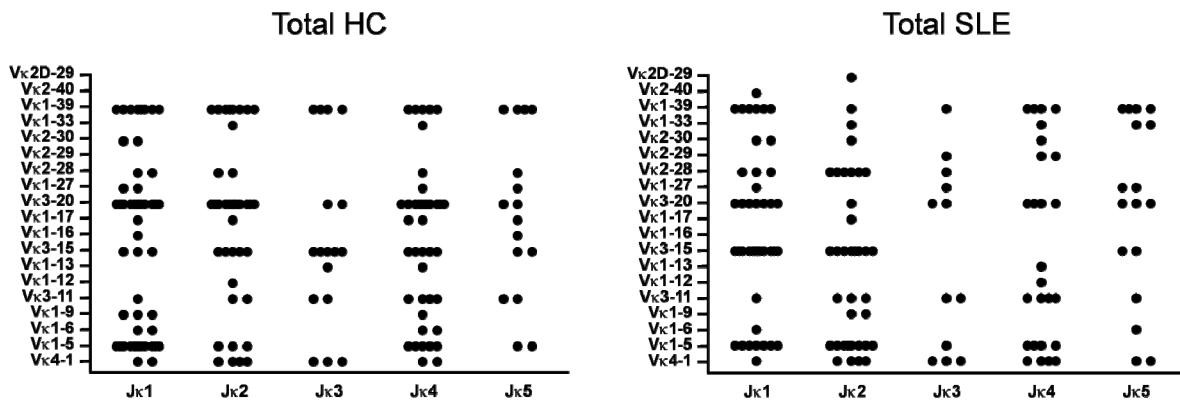


Figure 9: V κ to J κ joining in SLE patients and healthy controls. Dots indicate joining of V to J gene segments of single Ig κ L chains. The most proximal V and J gene segments are closest to the origin of each graph.

Signs of enhanced rearrangement on the Ig λ L chain locus have been reported in SLE (Dorner, et al., 1999). To determine if differences in secondary rearrangement on the Ig λ locus play a role in SLE IgG⁺ memory B cells, the joining of V λ to J λ of SLE patients and HC, as described above for the V κ to J κ joining, was analyzed. Since Ig λ does not rearrange by inversion, a clustering of joining events in the lower left or the upper right corner could be interpreted as a decrease or an increase in secondary rearrangement, respectively. Again, the joining events were comparable between HC and SLE, and there was no evidence for an alteration in the V λ to J λ joining in SLE (Figure 10). The frequency of J λ 3 joining to V λ 1-36 and above was similar (24% for HC and 20 % for SLE), and the increased presence of J λ 3 in SLE was also associated with an increased joining to downstream V λ gene segments.

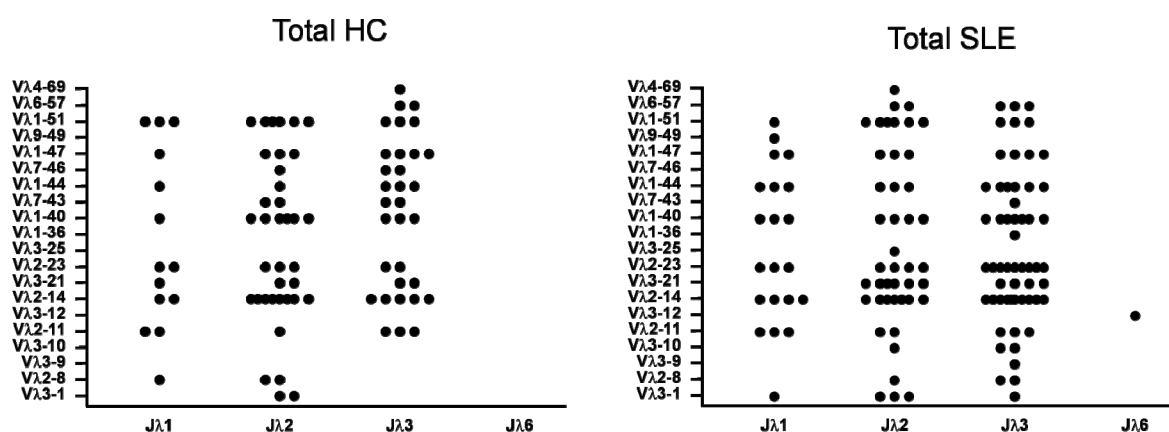


Figure 10: V λ to J λ joining in SLE patients and healthy controls. Dots indicate joining of V to J gene segments of single Ig λ L chains. Proximal V and J gene segments are closest to the origin of each graph.

Even though the primary rearrangement on the IgH chain locus deletes all the unrearranged D segments and a secondary rearrangement would contradict the 12-23 spacer rule, a number of studies has reported a VH replacement on the H chain locus (Chen, et al., 1995; Deane and Norton, 1990; Kleinfeld, et al., 1986; Reth, et al., 1986; Wilson, et al., 2000; Zhang, et al., 2003). The remaining nucleotides of the VH segment of the first rearrangement are not unambiguously distinguishable from N addition, and to determine if unusual rearrangements of the IgH chains were enriched in the SLE IgG⁺ memory B cell pool, the joining of VH to JH gene segments was analyzed. No general difference was observed between SLE patient and HC (Figure 11).

The majority of the VH to JH joining events was comparable between SLE patients and HC and differences resulted from insignificant general changes in the gene segment usage (for example a reduced expression of JH1 in SLE, 0.8% vs. 3.1% in HC; Figure 7A bottom and 11).

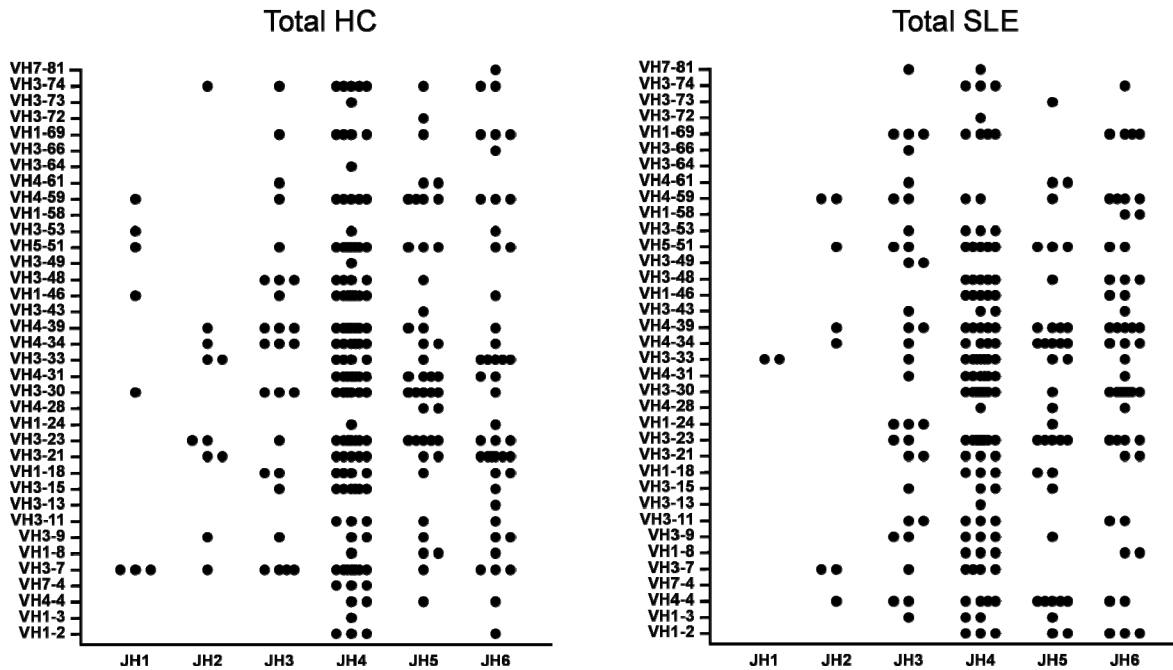


Figure 11: VH to JH joining in SLE patients and healthy controls. Dots indicate joining of V to J gene segments of single IgH chains. Proximal V and J gene segments are closest to the origin of each graph (Lefranc, et al., 2005).

3.3.5 IgG isotype subclass distribution

The IgG isotype subclasses have been attributed to different effector functions such as Fc γ receptor binding or activation of complement (Bindon, et al., 1988; Nimmerjahn and Ravetch, 2008). The subclasses expressed by the IgG⁺ memory B cells could be determined by analyzing the sequences of the 2nd PCR products (chapter 2.1.3 and (Tiller, et al., 2008)). Three of the four patients (SLE169, SLE175, and SLE176) showed a significant alteration in the distribution of their IgG subclasses ($P \leq 0.005$; Figure 12). IgG1 was increased in SLE175 (77.0% vs 66.7% in HC). SLE169, SLE175, and SLE176 all displayed a bias towards IgG3 (23.1%, 18.0%, and 24.6%, respectively vs. 6.3% in HC) and reduced expression of IgG2 (7.7%, 4.9%,

and 15.8%, respectively vs. 23.4% in HC). Only SLE174 exhibited the normal distribution dominated by IgG1 and IgG2 ($P=0.945$; Figure 12).

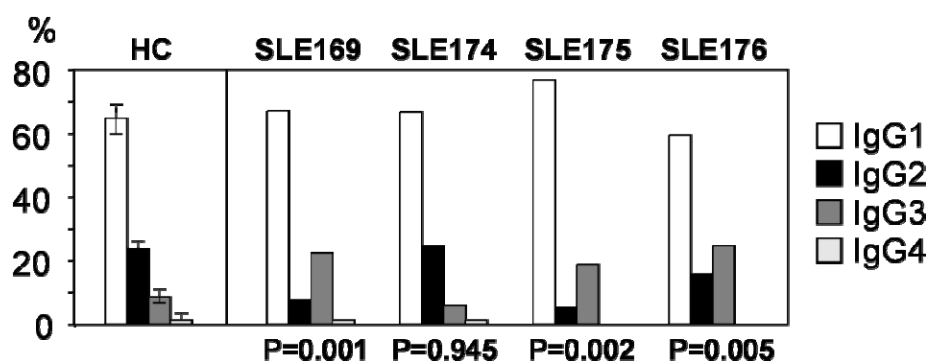


Figure 12: IgG isotype subclass distribution in IgG⁺ memory B cells of SLE patients and healthy controls. Bar graphs show IgG1 (white bars), IgG2 (black bars), IgG3 (dark gray bars) and IgG4 (light gray bars) subclass frequencies. P-values compare individual patients to healthy controls. Standard deviations are indicated for healthy controls.

3.3.6 Mutational frequency of IgVH and VL gene segments

The mutational frequency has been reported to be increased in SLE IgG⁺ memory B cells (Dorner, et al., 1999; Dorner and Lipsky, 2001). Therefore the number of nucleotide exchanges in the IgH and IgL V gene segments (VH, V κ , and V λ) cloned from the SLE patients was determined. A significant decrease was found in all V gene segments in comparison to HC. The average number of mutations in VH showed a reduction from 17.6 in HC to 11.2 in SLE ($P<0.0001$), in V κ the numbers dropped from 9.6 in HC to 5.6 in SLE ($P<0.0001$), and in V λ from 8.1 in HC to 5.4 in SLE ($P<0.0001$; Figure 13 and SI: Figure 29; (Tiller, et al., 2007)).

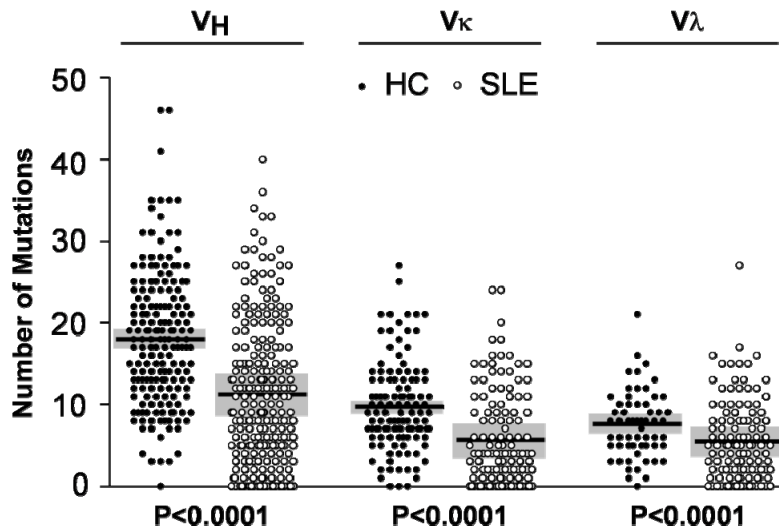


Figure 13: Number of somatic hypermutations in SLE patients and healthy controls. Dots represent individual V_H , V_K , and V_L genes of antibodies from IgG^+ memory B cells of four healthy donors (filled circles) and four SLE patients (open circles). Horizontal lines represent the average number of mutations and gray bars represent the standard deviation to show variability of mean values among individuals. P values are depicted comparing SLE patients and healthy controls.

The ratios of replacement (R) to silent (S) somatic hypermutations (SHM) in the FWRs and the CDRs of the IgV gene segments are an indicator for antigen-driven selection processes. To estimate if the observed reduction in the mutational frequency of the SLE IgG^+ memory B cells was linked to defects in selection, the R/S ratios of the SLE IgH and IgL V gene segments were compared to those of the HC. The lower frequency of SHM was not associated with considerable alterations in the R/S ratio in the SLE IgG^+ memory B cells (Figure 14). The absolute ratios between HC and SLE were comparable (2.1 vs. 2.0 for V_H , 1.9 vs. 1.9 for V_K , and 2.2 vs. 2.1 for V_L , respectively), and R/S ratios were generally higher in the CDRs than in the FWRs (2.3 to 6.7 vs. 0.9 to 1.8, respectively; Figure 14).

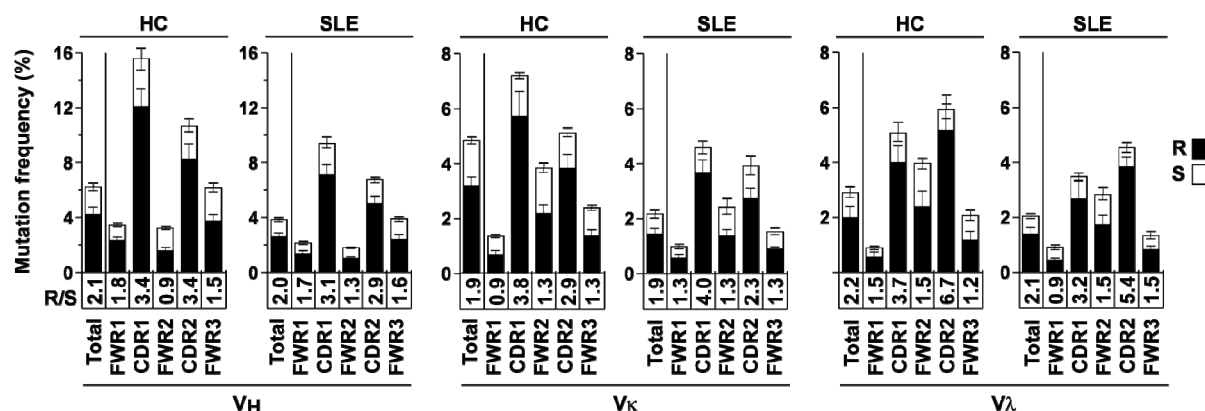


Figure 14: R/S ratios in SLE patients and healthy controls. Bar graphs indicate the frequency of R (black bars) and S (white bars) nucleotide exchanges per base pair in FWRs and CDRs in VH, VK, and VL gene segments. Standard deviations are indicated. The R/S ratio for each region is shown below the graphs.

The mutational frequency moreover did not correlate with the patients' age. For example, the youngest patients, SLE175, showed the highest average number of V gene SHM of all and the average number of VH gene SHM was lower in SLE174 than in the age-matched HC PN (10.5 vs 17.0; SI: Figure 29). The average number of mutations was generally higher in VH than in VL genes in HC and SLE (Figure 13). Strikingly, in SLE169 the ratio of VH to VL gene SHM was significantly increased (4.6 as compared to 2.4-2.7 in the other SLE patients, $P=0.005$, and 2.3-3.0 in HC, $P=0.001$; Figure 15 and SI: Table 5-9; (Tiller, et al., 2007)). The association of a mutated IgH with an unmutated IgL chain was further more frequently detected than in the other SLE patients or HC.

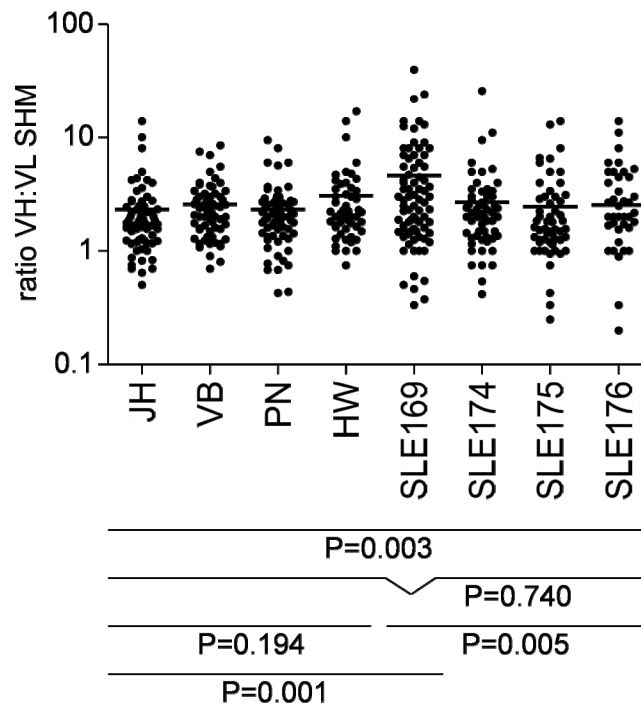


Figure 15: Ratios of VH to VL mutations. Dots show ratios of VH to VL mutations of single antibodies cloned from single IgG⁺ memory B cells from healthy donors JH, HW, PN, and VB and SLE patients 169, 174, 175, and 176. Absolute numbers of V gene segment FWR1-FWR3 nucleotide exchanges as compared to germline were used for the calculation. In cases where mutated IgH chains were associated with unmutated IgL chains (Tables 5.1-5), VL mutation values were artificially set from 0 to 1. P-values indicate variance and were calculated using 1 way ANOVA analysis. Horizontal lines represent mean values.

In summary, antibodies cloned from IgG⁺ memory B cells of SLE patients showed a decrease in SHM, an increase in Ig λ chain usage, alteration in the IgV κ 2 gene family and IgV κ 3-20 gene segment usage, whereas abnormalities in Ig gene features such as IgDH usage, IgH CDR3 length, IgV λ usage, and IgG isotype subclass distribution were variable among patients.

3.4 Polyreactive and self-reactive IgG memory B cells antibodies in SLE

3.4.1 Polyreactivity in IgG memory B cell antibodies

In healthy humans, substantial numbers of IgG⁺ memory B cells express self-reactive and polyreactive receptors (Koelsch, et al., 2007; Tiller, et al., 2007). To determine the frequency of polyreactive IgG⁺ memory B cells in patients with SLE, the 200 recombinant antibodies were tested for reactivity against ssDNA, dsDNA, insulin, and LPS. Antibodies reactive to at least two of these structurally diverse self and non-self antigens were considered polyreactive. Polyreactive IgG⁺ memory B cells were found in all the SLE patients but the frequency of such cells was not significantly different from HC (26% SLE169, 18% SLE174, 15% SLE175, and 21% SLE176 as compared to 23% for four HC; Figure 16; (Tiller, et al., 2007)). Further, most of the antibodies revealed only low levels of polyreactivity. A small number of highly polyreactive antibodies was found in SLE169, but not in the other patients. Thus, the general frequency of polyreactive IgG⁺ memory B cells in SLE was not significantly different from HC (23% in healthy vs. 22% in SLE, $P=0.711$; Figure 16; (Tiller, et al., 2007)).

Since CDR3 length has been associated with polyreactivity (Crouzier, et al., 1995; Ichiyoshi and Casali, 1994; Klonowski, et al., 1999), it was assessed comparing polyreactive and non-polyreactive antibodies of SLE patients and HC. While the CDR3 length was significantly increased in polyreactive antibodies cloned from SLE patients, this was not the case for the HC (15.2 vs. 13.8 in SLE, respectively, $P=0.037$ and 13.7 vs 13.8 in HC, respectively, $P=0.854$, SI Table 5-9 (Tiller, et al., 2007)).

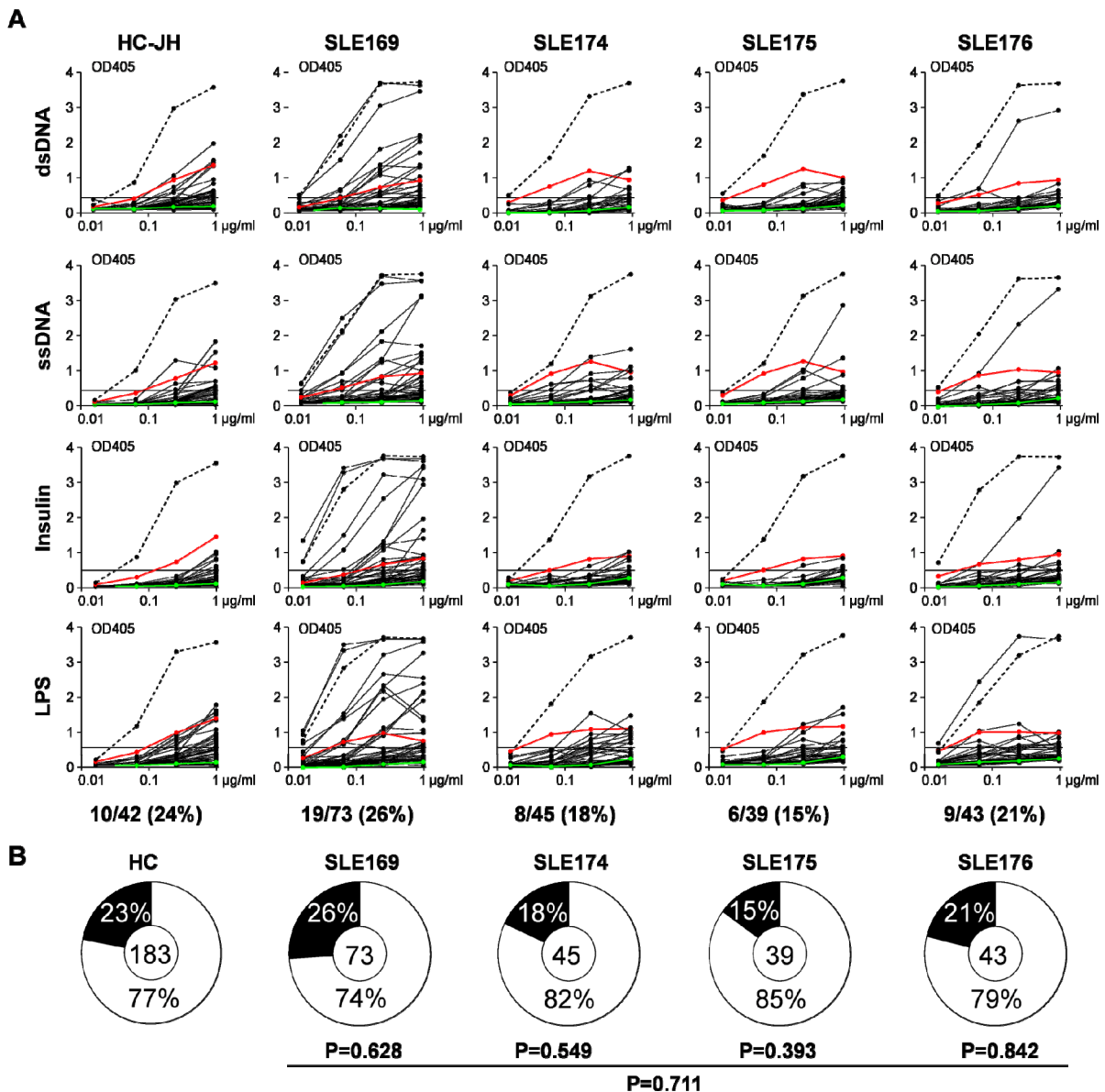


Figure 16: Polyreactivity of IgG memory B cell antibodies from SLE patients. IgG memory B cell antibodies from healthy donor JH and patients with SLE were tested for polyreactivity by ELISA with dsDNA, ssDNA, LPS, and insulin. (A) Dotted lines represent the high positive control antibody ED38 (Meffre, et al., 2004). Horizontal lines show cut-off OD405 for positive reactivity. Green and red lines show the negative control antibody mGO53 and low positive control antibody eiJB40, respectively (Wardemann, et al., 2003). (B) Pie charts summarize the frequency of polyreactive (black) and non-polyreactive (white) IgG⁺ memory B cell clones from three published (Tiller, et al., 2007) and one additional healthy control (HC-JH) and of the individual SLE patients. The number of tested antibodies is indicated in the center of each pie chart. P-values are in comparison to the four healthy controls (Tiller, et al., 2007).

3.4.2 Self-reactivity in IgG memory B cell antibodies

HEp-2 cell ELISA, enriched for nuclear antigens, and indirect immunofluorescence assay (IFA) are clinical tests to detect ANAs, which are a hallmark feature of SLE (Egner, 2000). To determine the frequency of ANAs produced by IgG⁺ memory B cells in SLE, all 200 purified recombinant antibodies were tested by HEp-2 cell ELISA and IFA. Surprisingly, no general increase in self-reactivity as compared to HC was found (on average 33% in HC vs. 30% in SLE; Figure 17; (Tiller, et al., 2007)). Additionally, the IFA staining patterns of the recombinant SLE antibodies were indistinguishable from HC showing diverse nuclear and cytoplasmic staining patterns (Figure 17B and C; (Tiller, et al., 2007)). Nevertheless, two antibodies (107, 128) cloned from SLE175 exhibited a high level of self-reactivity in the HEp-2 cell ELISA, that had not been observed in any of the other SLE patients or HC. These two antibodies further differed from other self-reactive antibodies in the ELISA, such as gp103 κ from SLE169, in that they did not show any sign of reactivity in the polyreactivity ELISA (Figure 17A and SI: Table 5 and 7).

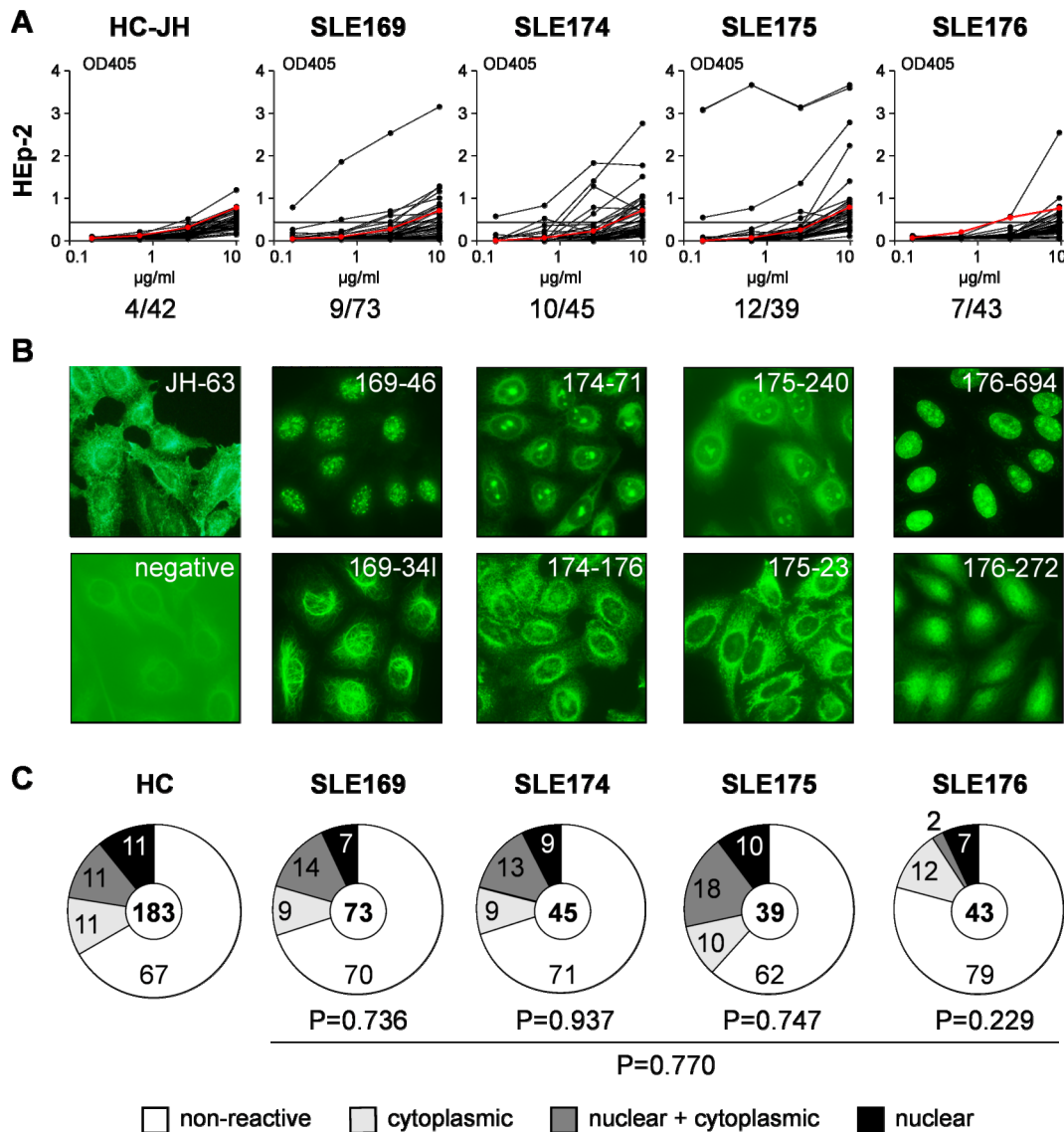


Figure 17: Self-reactive antibodies in the SLE IgG+ memory B cell pool. (A) IgG memory B cell antibodies from healthy control JH and patients with SLE were tested for self-reactivity by HEp-2 cell ELISA. Horizontal line shows ELISA cut-off OD405 for positive reactivity and red line shows low positive control serum. For each individual the number of HEp-2 cell self-reactive antibodies out of all antibodies tested is shown below the graphs. (B) IFA shows typical HEp-2 cell staining patterns of antibodies cloned from IgG+ memory B cells of healthy donor JH and patients with SLE. Staining pattern of a non-self-reactive antibody (negative) by IFA and HEp-2 ELISA is shown for comparison. (C) Pie charts summarize the frequency of self-reactive IgG memory B cell antibodies from healthy controls and SLE patients with nuclear (black), nuclear plus cytoplasmic (dark gray), and cytoplasmic (light gray) HEp-2 cell IFA staining patterns, and the frequency of non-reactive antibodies (white). The number of tested antibodies is indicated in each pie chart center. P-values are in comparison to IgG memory B cell antibodies from four healthy controls (Tiller, et al., 2007).

3.4.3 SLE IgG⁺ memory B cells producing anti-Ro and anti-La specific antibodies

In order to determine whether any of the recombinant SLE antibodies showed specificity for SLE-associated autoantigens and to identify the cognate antigens of the two antibodies cloned from SLE175 (107, 128) that were strongly self-reactive in the HEp-2 cell ELISA, they were tested on a line immunoblot assay (LIA). LIA is a clinical assay, which enables to simultaneously test for reactivity against 13 purified SLE antigens on a membrane. For comparison an additional number of 84 recombinant antibodies cloned from HC was tested. From the 200 SLE antibodies tested, six showed reactivity against the ribonuclear proteins Ro/SSA and La/SSB in this assay, including antibodies 107 and 128, and none of the antibodies from the HC were reactive. All of the reactive antibodies came from patient SLE175, which had serum titers of the same specificities in this assay (Figure 18A). None of the antibodies were clonally related (SI: Table 7). Two antibodies (107, 128) were reactive against Ro52 and four were reactive with La (29, 162, 264, 276). One anti-La antibody further exhibited low cross-reactivity with Ro60 (29). Ro and La reactivity was further confirmed in an ELISA and with surface plasmon resonance with purified Ro and La, respectively (Figure 19 and data not shown). The anti-Ro and anti-La autoantibodies were encoded by uniquely rearranged IgH and Igκ L chain genes (SI: Table 7). They were highly mutated and the mutation frequency was significantly increased in comparison to the other IgG memory B cell antibodies cloned from patient SLE175 ($P=0.003$; SI: Figure 29 and Table 7). Although the patients' sera displayed reactivity against dsDNA, RNP, or Sm, specific antibodies to these antigens among the 200 tested were not identified. In summary, a high frequency of IgG⁺ memory B cells producing SLE antibodies (6/39, 15%) was found in one of the four patients, and these antibodies reflected the specificity of serum autoantibodies measured in this patient (Figure 18 and Table 3).

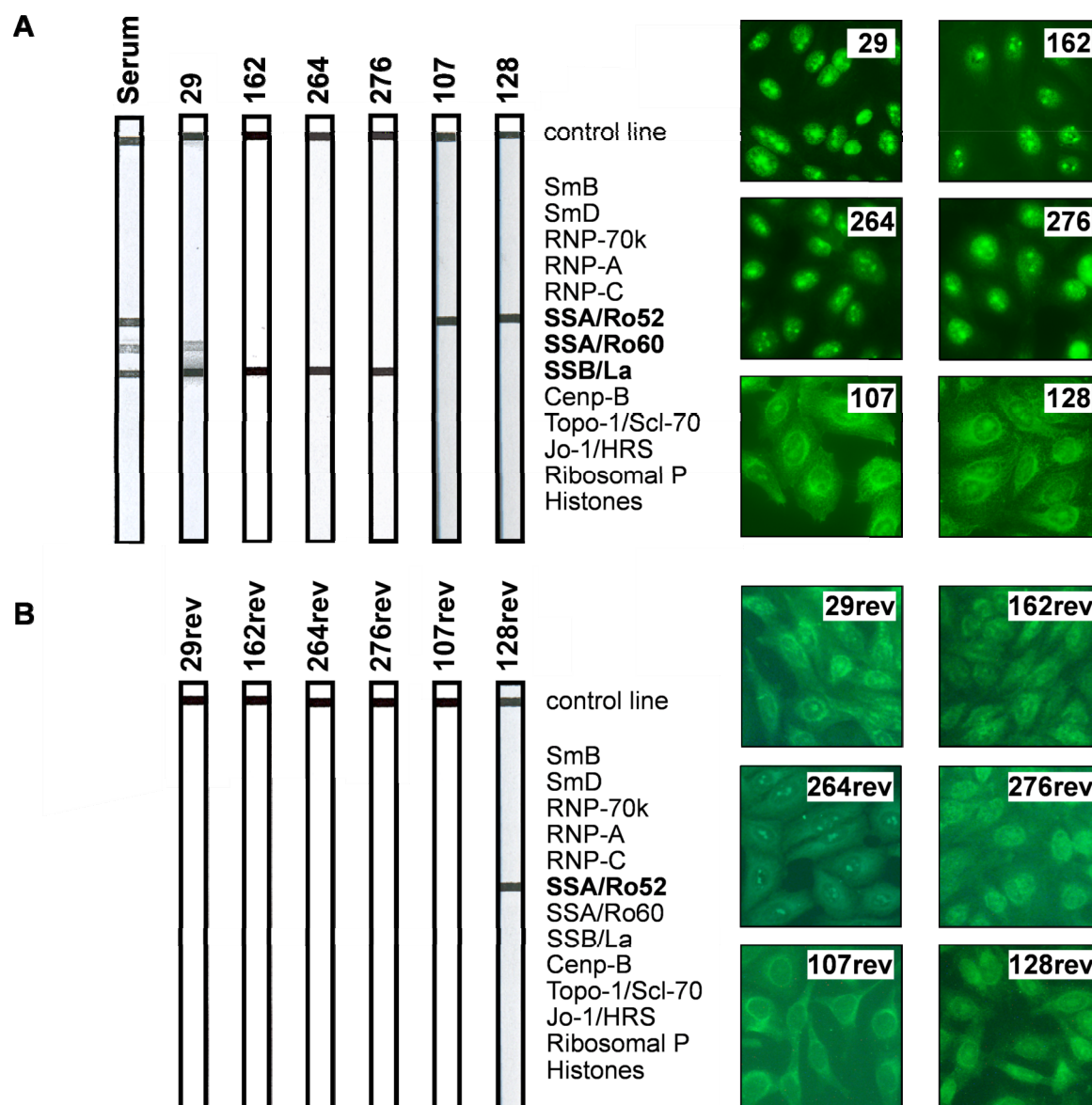


Figure 18: Ro52/SSA-reactive and La/SSB-reactive SLE IgG memory B cell antibodies. (A) Line immunoblot assays with 13 SLE-associated autoantigens (SmB, SmD, RNP-70k, RNP-A, RNP-C, Ro52/SSA, Ro60/SSA, La/SSB, Cenp-B, Topo-1/Scl-70, Jo-1/HRS, Ribosomal P, Histones) identified four La/SSB-reactive (29, 162, 264, 276) and two Ro52/SSA-reactive (107, 128) antibodies among 200 tested IgG memory B cell antibodies from SLE patients and 84 tested IgG memory B cell antibodies from healthy controls. Anti-Ro52/SSB and anti-La/SSB antibodies were all from SLE175. LIA result obtained with serum from the same patient is shown for comparison. HEp-2 cell IFA staining patterns of the same antibodies are shown. (B) Germline versions of the two anti-Ro52/SSA and four anti-La/SSB reactive IgG memory B cell antibodies from SLE175 were tested by LIA and IFA as in (A).

3.4.4 Reversion of mutations of anti-Ro and anti-La specific autoantibodies

Numerous studies report autoantibody specificities to be generated by SHM through the introduction of new aa residues (Radic, et al., 1993; Wellmann, et al., 2005). To determine if anti-Ro52 and La-reactivity was due to SHM, a PCR-based strategy was applied to revert the mutated IgH and IgL chain genes of the six anti-Ro52 and anti-La antibodies to their germ-line sequence (Figure 5). Thereafter, the reverted antibodies were compared for their reactivity in our HEp-2 cell ELISA, IFA, and LIA to their mutated counterparts. In the absence of SHM five of six antibodies lost reactivity in HEp-2 cell ELISA, IFA, LIA, and ELISA with purified Ro and La, respectively (Figure 18B and 19). Only one of the germ-line antibodies, 128rev, retained a low level of HEp-2 cell reactivity in the ELISA and IFA. The same antibody was also reactive with Ro52 in the LIA and ELISA with purified Ro. To determine if the anti-Ro and anti-La antibodies arose from polyreactive precursors, the reverted antibodies were tested in the polyreactivity-ELISA. Only 128 showed unspecific polyreactivity in the germ-line form (SI: Table 11).

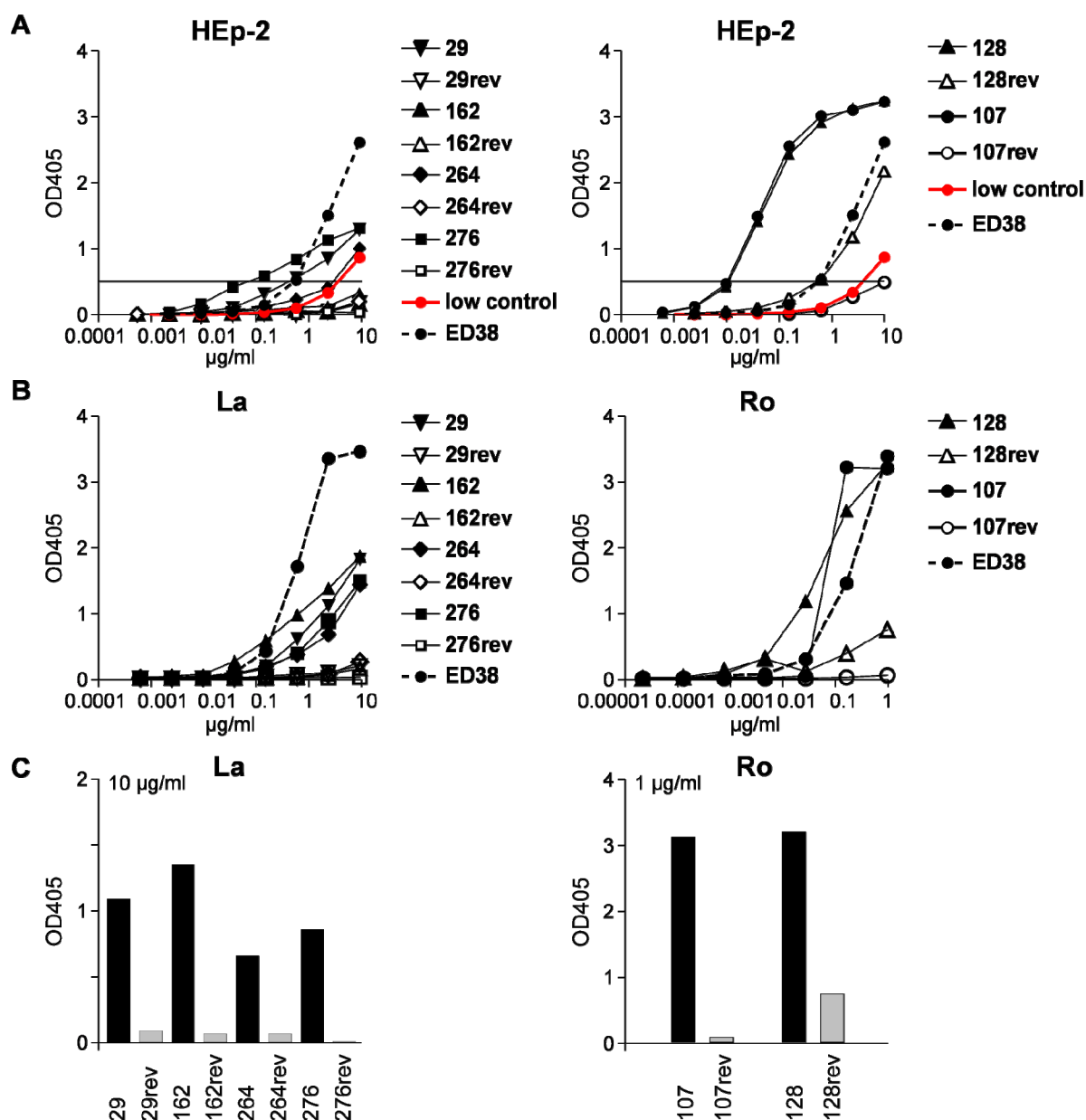


Figure 19: Ro52/SSA-reactive and La/SSB-reactive SLE IgG memory B cell antibodies. Results of HEp-2 ELISA (A) and ELISA with purified recombinant Ro and La protein (B) and (C) of recombinant mutated (filled symbols) anti-La/SSB (29, 162, 264, 276; left graph) and anti-Ro52/SSA (107, 128; right graph) antibodies and their germline counterparts (open symbols). Red lines in the HEp-2 ELISA graphs represent low positive control serum (A), dotted lines represent positive control antibody ED38 (Meffre, et al., 2004) and horizontal lines show cut-off OD₄₀₅ for positive reactivity (A), (B). Bar graphs show OD₄₀₅ of mutated (black) and reverted (gray) anti-La/SSB and anti-Ro/SSA antibodies at 10 or 1 µg/ml, respectively (C).

To confirm the role of SHM in the generation of Ro52 and La reactive antibodies, an additional number of 21 randomly selected mutated SLE IgG memory B cell antibodies was reverted to their germ-line form and tested for polyreactivity and self-reactivity by ELISA and IFA. The majority of the mutated polyreactive and self-reactive antibodies lacked reactivity in their germ-line form (8/11, 72.7% and 12/18, 66.7%, respectively; Figure 20A and B, and SI: Table 11). Only a few antibodies showed self-reactivity and polyreactivity independent of SHM (5/27, 18.5% and 4/27, 14.8%, respectively), including 128rev with specificity for Ro52 in the mutated form. The germ-line form of all other Ro52 and La reactive antibodies lacked polyreactivity (Figure 20 and SI: Table 11).

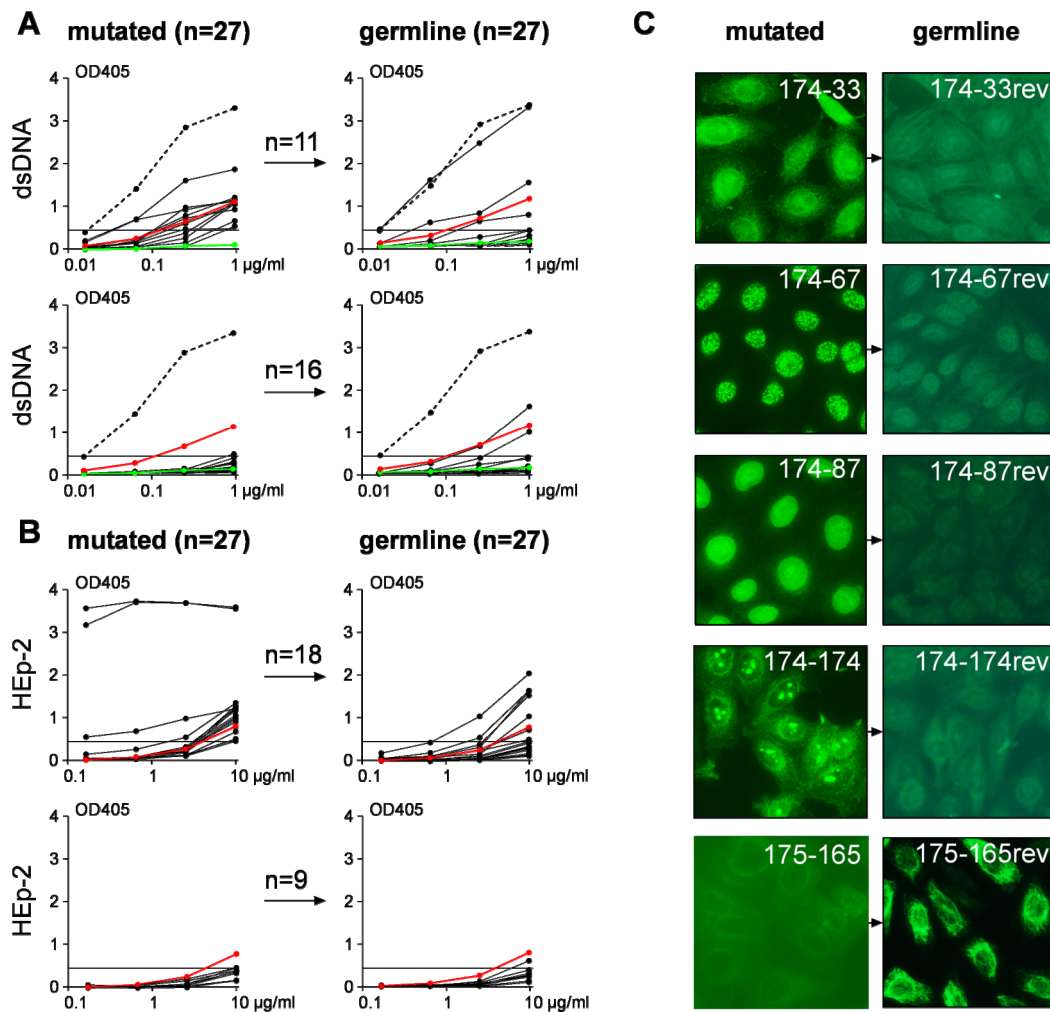


Figure 20: Somatic hypermutation contributes to poly-reactivity and self-reactivity in IgG memory B cell antibodies in SLE. (A) IgH and IgL chains from IgG memory B cell antibodies of SLE patients were reverted into their germline counterparts by PCR. Recombinant mutated (left) IgG memory B cell antibodies and their germline counterparts (right) were tested for polyreactivity (A) and self-reactivity by HEp-2 cell ELISA (B) and IFA (C). (A) Polyreactive mutated (top left) antibodies and mutated non-polyreactive (bottom left) antibodies were tested for polyreactivity by ELISA and compared to their germline counterparts (right). Representative polyreactivity graphs with dsDNA as antigen are shown. Dotted lines represent the high positive control antibody ED38 (Meffre, et al., 2004), red lines represent the low positive control antibody eiJB40 and green lines represent the negative control antibody mGO53 (Wardemann, et al., 2003). Horizontal lines show cut-off OD₄₀₅ for positive reactivity. (B) HEp-2 cell self-reactive mutated (top left) antibodies and mutated non-self-reactive (bottom left) antibodies were tested for HEp-2 cell self-reactivity by ELISA and compared to their germline counterparts (right). Red lines represent low ANA serum controls and horizontal lines show cut-off OD₄₀₅ for positive reactivity. (C) Typical HEp-2 cell IFA staining patterns of mutated IgG memory B cell antibodies (left) and their germline counterparts (right).

4 DISCUSSION

4.1 IgG⁺ memory B cells in patients with SLE

Abnormalities in the IgH and IgL chain repertoire of B cells in the autoimmune disease systemic lupus erythematosus have been reported (Bensimon, et al., 1994; Dorner, et al., 1999; Dorner and Lipsky, 2001; Manheimer-Lory, et al., 1997; Pugh-Bernard, et al., 2001; Suzuki, et al., 1996; Yurasov, et al., 2006; Yurasov, et al., 2005). The analysis performed in this study could confirm a great variability in Ig gene features and usage among such patients. All the analyzed DNA sequences of the 245 antibodies from the four SLE patients resulted from an independent V(D)J recombination. Massiv clonal expansion was therefore not a feature of SLE IgG⁺ memory B cells. The number of analyzed B cells, nevertheless, might have been too small to detect low levels of clonal expansion. A distinct CD38⁺CD27⁺⁺ plasmablast population, previously linked to SLE disease activity, was only identified in one of the four SLE patients further underlining the great variability in SLE features (Arce, et al., 2001; Jacobi, et al., 2003; Odendahl, et al., 2000; Vallerskog, et al., 2007).

4.2 The IgH chain repertoire in SLE IgG⁺ memory B cells

Significant differences in IgH gene usage were not found between the IgG⁺ memory B cells of SLE patients and HC and only observed in individual patients, such as an altered DH usage in SLE174 and VH usage in SLE169. Previously observed differences such as an increased VH3 usage could not be confirmed and might have resulted from a broader selection on IgD⁺ B cells or from the limited number of sequences analyzed in those studies (Odendahl, et al., 2000; Pugh-Bernard, et al., 2001).

A long IgH chain CDR3 has been associated with self- and polyreactivity, due to a greater flexibility in the protein structure of the ag-binding domain, which would result in decreased specificity (Crouzier, et al., 1995; Ichiyoshi and Casali, 1994; Klonowski, et al., 1999). Analysis of antibodies cloned from human progenitor B cells showed that the CDR3 length was significantly increased in these highly polyreactive

B cell compartments as compared to less polyreactive later developmental B cell stages (Wardemann, et al., 2003). The CDR3 length of SLE IgG⁺ memory B cells was not significantly different from HC, confirming an earlier study (de Wildt, et al., 2000). When analyzing the polyreactive SLE antibodies, the average IgH CDR3 length was significantly increased compared to that of the non-polyreactive SLE antibodies. This could, however, not be confirmed for the HC antibodies. Therefore, CDR3 length alone might enhance, but it does not determine polyreactivity.

A second feature of IgH chain CDR3s that has been suggested to predispose B cells for self-reactivity is the presence of charged aa. In SLE, especially the role of positively charged aa in DNA-binding has been investigated (Eilat, et al., 1988; Li, et al., 2000; Radic, et al., 1993). Arginine residues, for example, enhance the specificity to dsDNA by binding within the major groove of the DNA backbone (Seeman, et al., 1976). IgG⁺ memory B cells from SLE patients exhibited no significant difference in the number of positively or negatively charged aa in their CDR3s in comparison to HC, confirming the results of an earlier study (de Wildt, et al., 2000). Nevertheless, these features primarily applied to strongly anti-dsDNA specific antibodies that were not found among those cloned from SLE IgG⁺ memory B cells in this study.

Certain VH gene family members have been attributed to intrinsic self-reactivity such as VH4-34 or VH3-23 and have been reported to be predominantly found in autoantibody-expressing B cells (Leucht, et al., 2001; Pugh-Bernard, et al., 2001). Based on this observation, studies have been performed using anti-idiotypic specific antibodies as a way to determine self-reactivity (Cambridge, et al., 2008; Cappione, et al., 2005; Isenberg, et al., 1993). Nevertheless, a detailed analysis of IgVH gene usage in systemic autoimmune diseases already led Dörner et al. to the conclusion that there is no intrinsic autoreactivity within special VH gene segments and no specific VH gene segment selection (Dörner and Lipsky, 2001). Except for an overrepresentation of VH4-4, which was only found in patient SLE169, no differences in the expression of single VH gene segments could be observed between HC and SLE. Further, the frequency of self-reactive B cells expressing either VH4-34 or VH3-23 is comparable to the level of self-reactivity generally found in IgG⁺ memory B cells of SLE patients and HC (33% in VH4-34 or VH3-23 expressing B cells vs. 30% in SLE and 33% in HC; Figure 17 and SI: Table 5-9; (Tiller, et al., 2007)). Determining antibody reactivity based on idiotype alone might be misleading and should be con-

sidered carefully, especially in the case of analyzing somatically mutated B cells since mutations can interfere with the binding of anti-idiotypic antibodies (Zheng, et al., 2004). It should further be mentioned that those earlier reports analyzed the VH gene usage of autoreactive B cells and did not give a general overview on the B cell repertoire in SLE.

4.3 The IgL chain repertoire in SLE IgG⁺ memory B cells

The analysis of the SLE Ig κ L chain repertoire revealed an increase in V κ 2 usage, confirming an earlier study which also found an enhanced expression of V κ 2 gene family members in IgG⁺ memory B cells of SLE patients (de Wildt, et al., 2000). V κ 3 usage was further decreased in SLE IgG⁺ memory B cells, particularly the usage of V κ 3-20. Similarly, mature naïve B cells from untreated active SLE patients showed a lower frequency in V κ 3-20 usage than HC, but this did not reach statistical significance (Herve, et al., 2007; Wardemann, et al., 2003; Yurasov, et al., 2005). Thus, it remains to be determined whether alteration in V(D)J recombination or selection are responsible for these differences.

The Ig λ L chain repertoire of SLE IgG⁺ memory B cells was comparable to HC. Changes in the Ig λ L chain repertoire observed in an earlier study of a single SLE patient could not be confirmed (Dorner, et al., 1999). This might be due to individual variations only present in this particular SLE patient. Changes in Ig λ gene usage were further not observed in a more recent study on SLE IgG⁺ memory B cells and are therefore not a frequent feature (de Wildt, et al., 2000).

In general, the B cell repertoire can even vary strongly within the same individual (Yurasov, et al., 2006). Yurasov et al. observed significant changes when comparing the repertoire of SLE patients during active disease with that of the same individuals during clinical remission.

4.4 Secondary rearrangement in SLE IgG⁺ memory B cells

Receptor editing has been proposed to be the dominant mechanism in establishing self-tolerance and is mainly achieved by secondary rearrangement on the light

chain locus. Impairment in secondary IgL chain rearrangement in SLE and other systemic autoimmune diseases has been suggested to participate in the generation of autoantibodies (Bensimon, et al., 1994; Dorner, et al., 1998; Samuels, et al., 2005; Suzuki, et al., 1996; Yurasov, et al., 2006). While a reduction could impede mechanisms normally leading to the elimination of self-reactive receptors, an increase was proposed to participate in the formation of new self-reactive specificities because deletion by further rearrangements would no longer be possible (Zouali, 2008). In SLE IgG⁺ memory B cells, an alteration in secondary rearrangement could not be observed in the analysis of V κ to J κ and V λ to J λ gene segment joining in comparison to HC. A major defect in secondary rearrangement is therefore not a common feature of SLE IgG⁺ memory B cells. There was further no evidence for differences in V(D)J recombination between SLE IgG⁺ memory B cells and HC when analyzing the joining of V H to J H gene segments.

4.5 Increased Ig λ L chain usage in SLE IgG⁺ memory B cells

Evidence for the role of Ig λ as an editing L chain, that eliminates undesired specificities by replacing the Ig κ L chain of a self-reactive B cell receptor, comes from observations in mice (Retter and Nemazee, 1998). Retter et al. found that 47% of all Ig λ L chain expressing murine B cells contain a productively rearranged Ig κ locus (Retter and Nemazee, 1998). In humans, the Ig κ L chain locus is likewise rearranged before the Ig λ L chain locus, and approximately 60% of human B cells express Ig κ (Goossens, et al., 2001; Hieter, et al., 1981; Szczepanski, et al., 2000; Wardemann, et al., 2004). In patient SLE169, the Ig λ L chain antibodies of Ig κ ⁺Ig λ ⁺ double expressing B cells exhibited autoreactivity only in concordance with the respective Ig κ L chain antibody, and in vivo experiments showed that Ig λ is more effective than Ig κ in silencing self-reactive IgH chains (Wardemann, et al., 2004). All four SLE patients showed a relative increase in Ig λ L chain usage. The increase in Ig λ L chain expressing B cells in SLE might result from extensive receptor editing either through increased stimulation of an overactive immune system or as an attempt to eliminate self-reactive receptors.

4.6 Allelic inclusion in SLE IgG⁺ memory B cells

Only one patient, SLE169, showed a high frequency of Igκ⁺Igλ⁺ double positive cells. Allelic inclusion can silence autoantibodies by diluting out autoreactivity. This mechanism allows B cells that produce self-reactive antibodies to pass the central self-tolerance checkpoint and to participate in immune responses (Casellas, et al., 2007; Doyle, et al., 2006; Gerdes and Wabl, 2004; Li, et al., 2004; Liu, et al., 2005; Velez, et al., 2007; Witsch, et al., 2006). In patient SLE169, one third of the antibodies of Igκ⁺Igλ⁺ double expressing B cells exhibited autoreactivity only with the respective Igκ L chain. Allelic inclusion thus contributed to the presence of IgG⁺ memory B cells expressing self-reactive antibodies. Double producing B cells were rare in the IgG⁺ memory B cell pool of the other SLE patients and were not enriched in naïve B cells from untreated SLE patients with active disease (Yurasov, et al., 2006; Yurasov, et al., 2005). Thus, isotypic inclusion is not a frequent feature of this disease. Nevertheless, it may be characteristic of a subgroup of SLE patients where it contributes to the presence of self-reactive B cells.

4.7 Somatic mutations and selection in SLE IgG⁺ memory B cells

IgG⁺ memory B cells from SLE patients showed overall significantly reduced numbers of IgH and IgL chain SHM. IgV gene R to S ratios in FWRs and CDRs were normal, suggesting that selection of activated B cells is not grossly affected. Others have reported an increase in Ig mutational frequency in peripheral CD19⁺ B cells in SLE (Dorner, et al., 1999; Dorner and Lipsky, 2001; Jacobi, et al., 2000), including skewed R to S ratios (Dorner, et al., 1999). These findings were not confirmed in another study on circulating IgG⁺ B cells, where, in fact, SHM frequency was found to be slightly lower in patients as compared to HC (de Wildt, et al., 2000). On average, the number of VH gene mutations was generally higher than in IgVL chain genes in SLE and HC and comparable to the values reported by others (Brezinschek, et al., 1998; de Wildt, et al., 1999; de Wildt, et al., 2000). Only patient SLE169 exhibited an increased IgVH to IgVL chain mutation ratio. The mutational frequency in Igλ L chain genes was lower than in Igκ L chain genes, as also observed by others (Tiller, et al., 2007).

4.8 IgG isotype subclass distribution was shifted in three SLE patients

A shift in the distribution of the IgG isotype subclasses was found in the IgG⁺ memory B cells from three of the four SLE patients. Altered IgG subclass frequencies have previously been noted in the sera of SLE patients (Oxelius, 1984; Tokano, et al., 1988). Since the shift in IgG isotype subclasses was neither consistent in the IgG⁺ memory B cells of the four patients considered in this study, nor in the SLE sera analyzed in the earlier reports, it is not a frequent feature of SLE.

A reduction of complement C3 and C4 was observed in two of the patients (SLE169 and SLE176), who also exhibited the highest levels of IgG3. IgG3 is the strongest activator of complement among Ig isotypes (Bindon, et al., 1988). The shift in IgG subclass distribution was observed in IgG⁺ memory B cells and their participation to antibody serum titers is not known. Yet, a strong correlation between memory B cells and antibody serum titers has been observed (Bernasconi, et al., 2002). A possible link between a shift in IgG isotype subclasses and the often reported reduced levels of complement components in SLE should be further investigated.

4.9 Polyreactivity in SLE IgG⁺ memory B cells

Due to a defect in early B cell self-tolerance checkpoints, patients with SLE show high numbers of circulating self-reactive and polyreactive mature naïve B cells (Yurasov, et al., 2006; Yurasov, et al., 2005). Polyreactive and self-reactive B cells can be recruited into germinal center reactions and contribute to the formation of autoreactive plasma cells and memory B cells. However, the frequency of self- and polyreactive IgG⁺ memory B cells in SLE was comparable to HC and suggests that the increased number of self-reactive naïve B cells does not have a strong impact on the general level of autoreactivity in the IgG⁺ memory B cell pool. The reversion experiments performed in this study further showed that self-reactive B cells mostly originated from non-reactive precursors. Thus, the defects that lead to abnormalities in IgG⁺ memory B cell tolerance in SLE may be independent of the earlier defects in tolerance. Nevertheless, it seems possible that certain autoantibody specificities are

more easily generated from a larger pool of self-reactive naïve B cells or directly selected into the antibody-secreting plasma cell pool.

4.10 Autoreactivity in SLE IgG⁺ memory B cells

IgG autoantibodies with high levels of reactivity for SLE autoantigens Ro and La were only identified in patient SLE175, where they made up a substantial fraction of the circulating IgG⁺ memory B cell pool. Because of the relatively small number of cloned antibodies from the other patients it is not possible to determine whether autoreactive memory B cells were infrequent or simply not circulating in the blood streams of these patients.

Ro- and La-reactive autoantibodies from patient SLE175 were highly mutated, and similar to low-level self-reactive and polyreactive antibodies from healthy donors, reversion to their germ-line form left them less or non-reactive, suggesting a competitive selection process. These observations are consistent with previous analyses showing that anti-DNA autoantibodies from SLE patients and mice acquire their reactivity through somatic mutations and are products of selection (Cocca, et al., 2001; Li, et al., 2003; Radic and Weigert, 1994; Wellmann, et al., 2005). Of all the reverted antibodies, only a few showed self- and polyreactivity independently of somatic mutations, confirming the results of previous reversion experiments (Herve, et al., 2005; Tiller, et al., 2007; Tsuiji, et al., 2006).

4.11 The role of memory in SLE

Within the past years, B cell depletion therapy applying the chimeric monoclonal anti-CD20 antibody Rituximab, originally developed against B cell lymphomas, has become an alternative approach in the treatment of autoimmune diseases. Due to the broad target molecule expression in the B cell lineage, it does not only deplete malignant but also normal B cells and has therefore been applied to patients that were nonresponsive or poorly responsive to conventional immunosuppressive therapy (Lu, et al., 2009; Tokunaga, et al., 2005). Responses to Rituximab treatment are variable. Some patients experience long-term clinical remission, while others exhibit

only short clinical remission or no response at all (Anolik, et al., 2004; Anolik, et al., 2007; Looney, et al., 2004). Studies on the dynamics of the repopulation of circulating B cells after depletion therapy have emphasized the role of memory B cells in systemic autoimmune diseases. In SLE and RA, the reappearance of high numbers of circulating CD27⁺ memory B cells has been correlated with a higher relapse rate and a limited time of clinical remission (Anolik, et al., 2007; Leandro, et al., 2006). It could however not be determined whether this was due to a fast regeneration or an efflux of nondepleted memory B cells from secondary lymphoid organs. In animal studies on cynomolgus monkeys and mice transgenic for human CD20, B cell depletion in lymphoid organs is not complete after Rituximab-treatment and varies between individuals, even if the same dose is applied (Gong, et al., 2005; Reff, et al., 1994; Schroder, et al., 2003). For humans, it has also been concluded that memory B cells persist in solid lymphoid tissue, where they survive depletion therapy (Pers, et al., 2007). Varying effects on B cells during ablative therapy is evident by a discrete response of certain autoantigen specificities. While anti-DNA is readily depleted from serum anti-Ro is not (Isenberg, et al., 1997; Leandro, et al., 2005; Ng, et al., 2007). Since B cell depletion therapy acts on structures independent of the variable region of the BCR, it is unlikely that B cells vary in their susceptibility to immunoablative therapy solely due to their receptor specificity, but rather that B cell responses differ according to certain self-antigens, for example due to differences in antigen-presentation. These findings therefore suggest that certain autoantigen-specific B cells arise through different mechanisms, are predominantly selected into different B cell compartments, or reside at different locations within the body.

The presence of baseline ENA, including Ro52 and La, further correlates with a faster reappearance of circulating CD27⁺ memory B cells after B cell depletion in SLE patients and thus a higher risk of relapse after treatment (Anolik, et al., 2007; Ng, et al., 2007). Therefore, autoantibody specificities should be considered in the decision on treatment. Since patients with a prolonged clinical remission were limited in their baseline autoantibody repertoire but anti-DNA positive, conventional markers in SLE such as anti-DNA titers, might be less relevant for disease severity and progression. Instead, the presence of certain specificities such as anti-ENAs should be taken more into account for the design of future therapies.

Continuous autoantibody titers, including anti-ENA, after B cell ablative therapy have been suggested to result from long-lived PCs, which do not express CD20 and are not depleted by standard therapy (Alexander, et al., 2009). Autologous stem cell transplantation has shown to induce remission in SLE, but it is only applied in severer cases due to the high mortality (Jayne, et al., 2004). Approaches especially targeting long-lived PCs have been undertaken in mice with the proteasome inhibitor Bortezomib (Neubert, et al., 2008). It decreases disease activity, even if application follows disease onset, and clinical trials for the application on human patients are on the way. Nevertheless, it can not be excluded that long-lived PCs are replenished by activated memory B cells that survived B cell depletion and reoccupy empty niches of depleted plasma cells. Memory B cells readily respond to activation by differentiation into antibody secreting B cells (Arpin, et al., 1997; Bernasconi, et al., 2002; Lanzavecchia, et al., 2006; Tangye and Good, 2007). This might especially be the case in an overactive immune system such as in SLE and with less competition for survival factors after depletion therapy (Desai-Mehta, et al., 1996; Lipsky, 2001).

4.12 Conclusion and outlook

The sequential analyses of the different B cell populations according to developmental stages would require a detailed analysis of the SLE plasma cell compartment as a next step. Antibody-secreting plasma cells reside in the bone marrow and should be the direct link to SLE serum autoantibodies titers. Single plasma cells from the bone marrow of SLE patients could be sorted and examined according to the method applied in this study.

In the present study, anti-Ro and anti-La antibodies were identified at high frequency within the IgG⁺ memory B cell compartment in one SLE patient. Anti-Ro and anti-La autoantibodies have been described as a marker for risk of congenital heart block in newborns of seropositive mothers (Lee, et al., 1994; Salomonsson, et al., 2002). Inhibitory effects have been confirmed in vitro on rat cardiomyocytes (Salomonsson, et al., 2005). For many SLE-associated autoantibody specificities, a role in pathogenesis has not been identified, which could enable a more directed from of treatment.

Possible ways of memory B cell activation and differentiation have further been discussed. Their contribution to antibody serum titers however, remains a substantial question.

Numerous checkpoints in B cell development have been identified (Tsuiji, et al., 2006; Wardemann, et al., 2003). In HC, self- and polyreactivity is increased in the IgG⁺ memory B cell compartment as compared to mature naïve B cells, suggesting no further checkpoint. Nevertheless, highly self-reactive B cells were found in one SLE patient. The question remains whether a checkpoint exists, excluding only highly self-reactive B cells from progressing into the IgG⁺ memory pool in healthy individuals that is defective in SLE patients, or whether another predisposition is required for the generation of highly self-reactive B cells in SLE. For this, a detailed analysis of GC B cell subsets would be necessary that compares the reactivity of B cells that acquired somatic mutations instantaneously, but were not yet subjects to possible selection mechanisms, with unmutated B cells and those about to leave the GC.

The lack of anti-DNA specific autoantibodies in the IgG⁺ memory B cell compartment raises the question if B cells are recruited into different compartments according to specificities, for example due to differences in antigen presentation. Since it is not possible to monitor this question within humans, it would be thinkable to immunize for example mice with structurally different antigens and afterwards assess different B cell compartments for the presence of ag-specific B cells.

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SUPPLEMENTAL INFORMATION

Supplemental figures

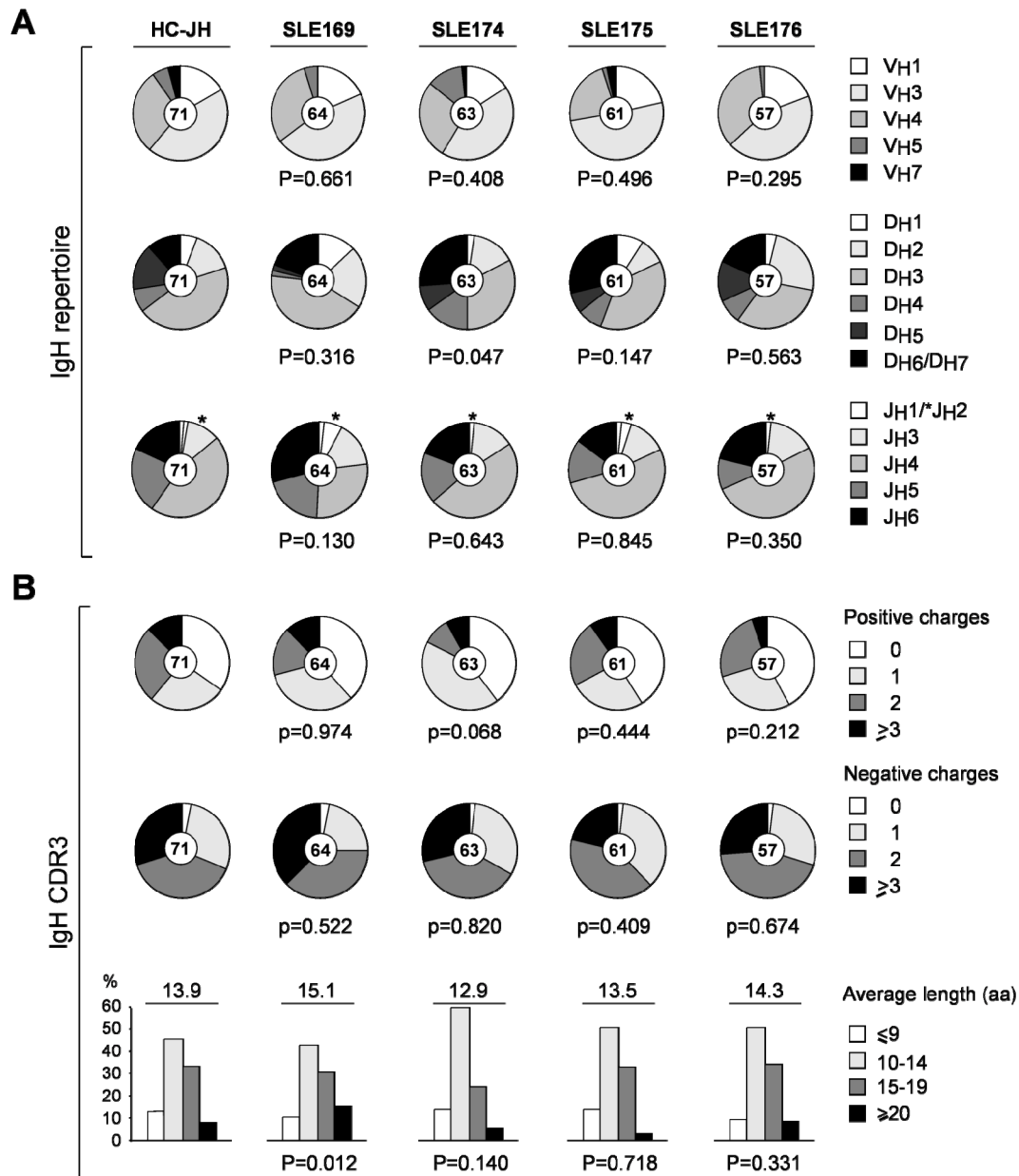


Figure 21: IgH chain V, D, and J gene usage and CDR3 region features of IgG memory B cell antibodies from healthy controls and SLE patients. IgH chain gene repertoire and CDR3 features of antibodies from IgG⁺ memory B cells of one HC donor (JH) and four patients with SLE (SLE169, SLE174, SLE175, SLE176). P-values are in comparison to combined data from HC-JH and three additional previously published healthy controls (Tiller 2007). (A) Pie charts depict V, D, and J family usage and (B) the proportion of IgH CDR3s with 0, 1, 2, or ≥ 3 positive charges. Bar graphs show frequencies of IgH CDR3s with ≤ 9 aa (white bars), 10–14 aa (light gray bars), 15–19 aa (dark gray bars), and ≥ 20 aa (black bars). The absolute number of sequences analyzed is indicated in the center of each pie chart. Average IgH CDR3 aa length is indicated above the bar graphs.

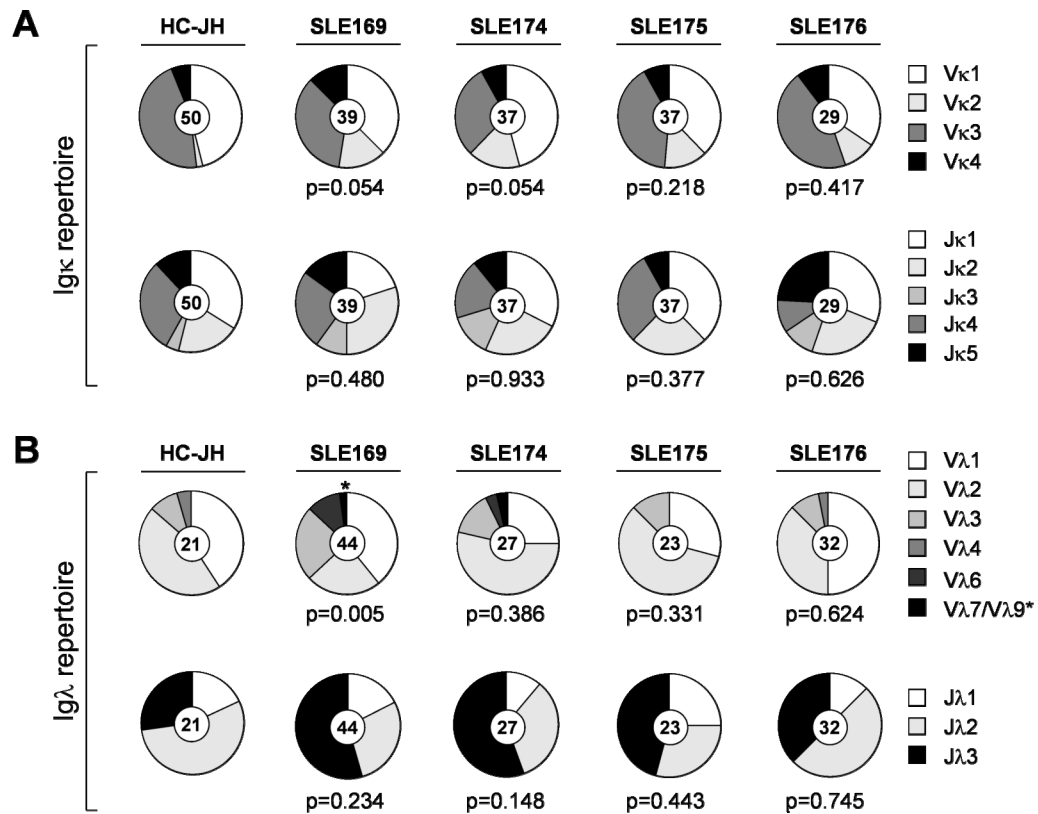


Figure 22: IgL chain V and J gene usage of IgG memory B cell antibodies from healthy controls and SLE patients. Pie charts depict V κ /J κ (A) and V λ /J λ gene family usage (B) of antibodies from IgG⁺ memory B cells of one HC donor (JH) and four patients with SLE (SLE169, SLE174, SLE175, SLE176). P-values are in comparison to combined data from HC-JH and three additional previously published healthy controls (Tiller 2007). The absolute number of sequences analyzed is indicated in the center of each pie chart.

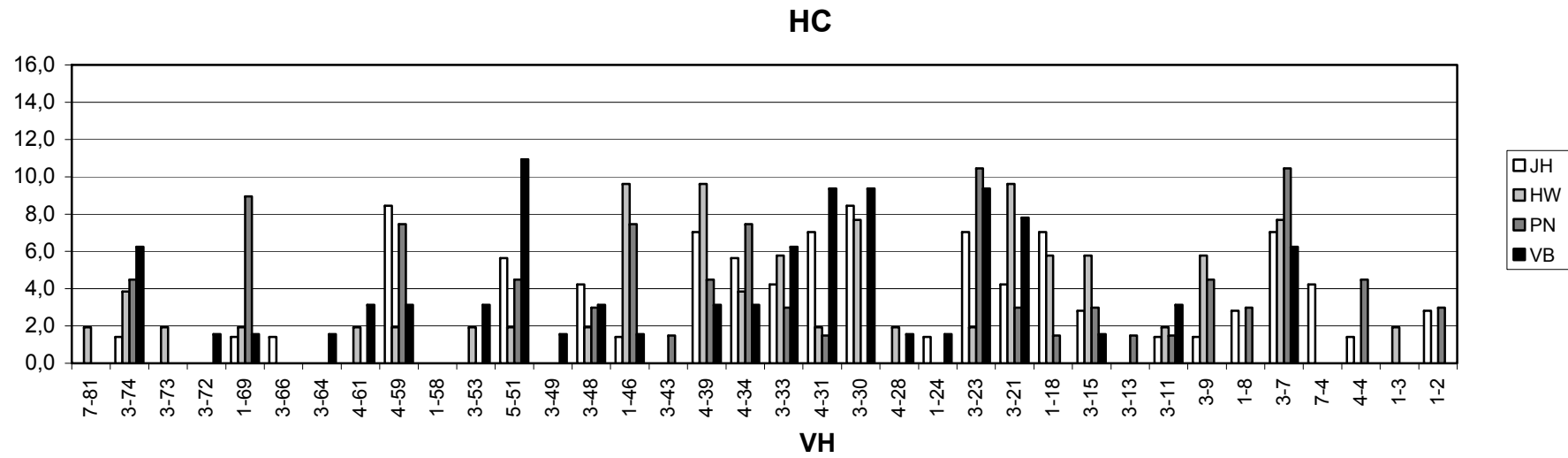


Figure 23: VH gene usage in IgG⁺ memory B cells of healthy donors JH, HW, PN, and VB. The frequency of individual VH genes is shown for each individual.

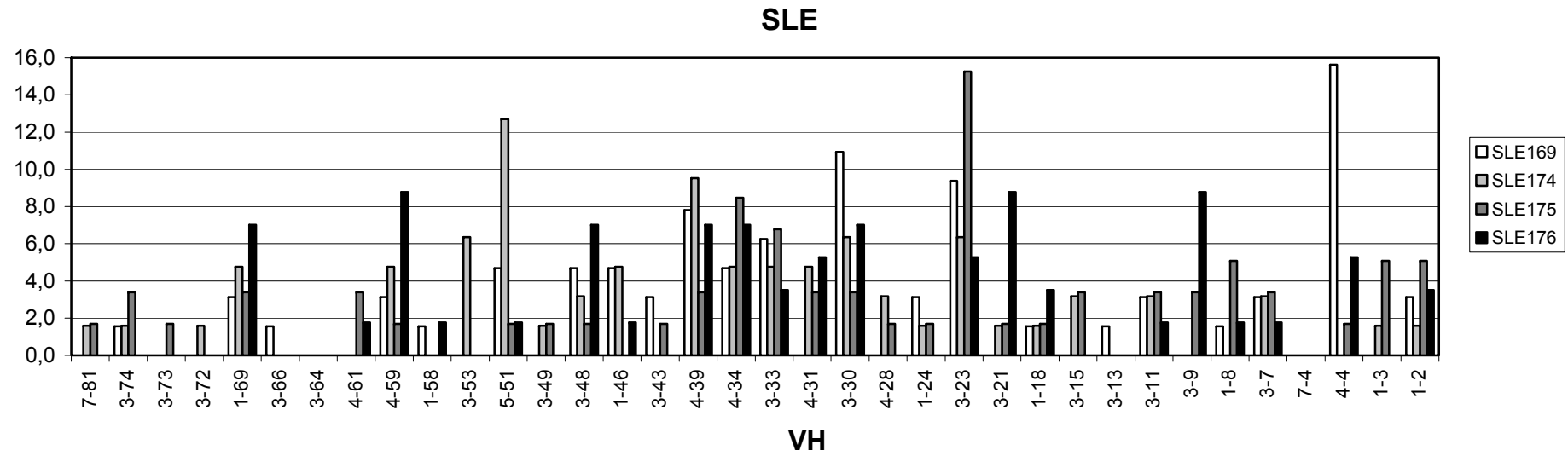


Figure 24: VH gene usage in IgG⁺ memory B cells of SLE patients 169, 174, 175, and 176. The frequency of individual VH genes is shown for each individual.

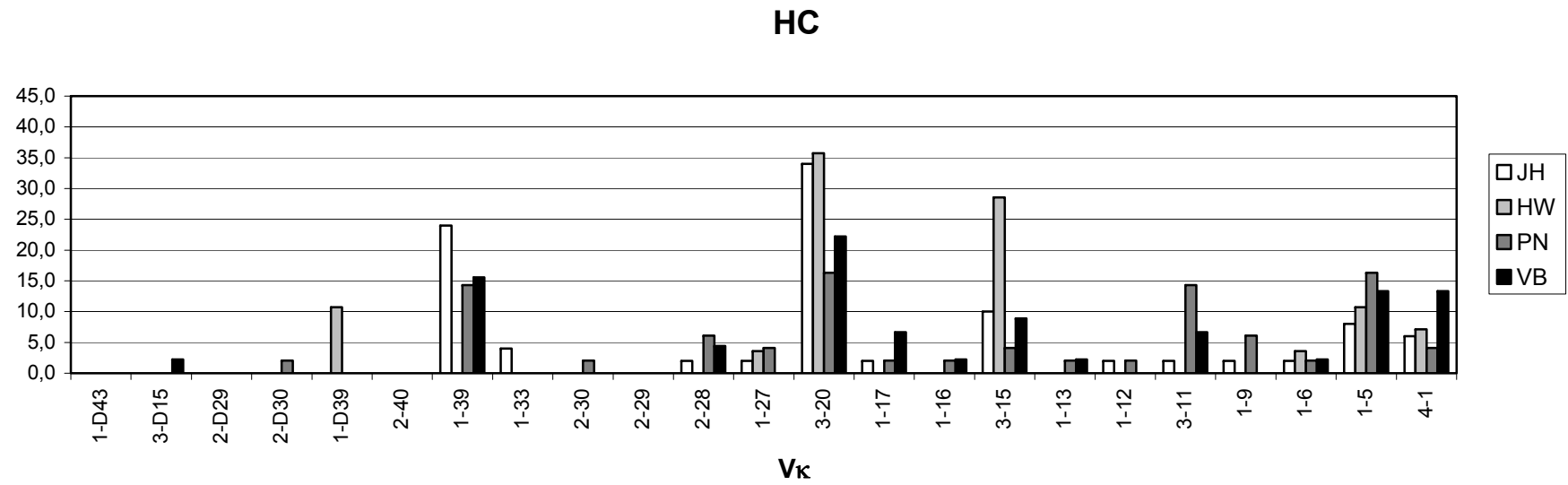


Figure 25: Vκ gene usage in IgG⁺ memory B cells of healthy donors JH, HW, PN, and VB. The frequency of individual Vκ genes is shown for each individual.

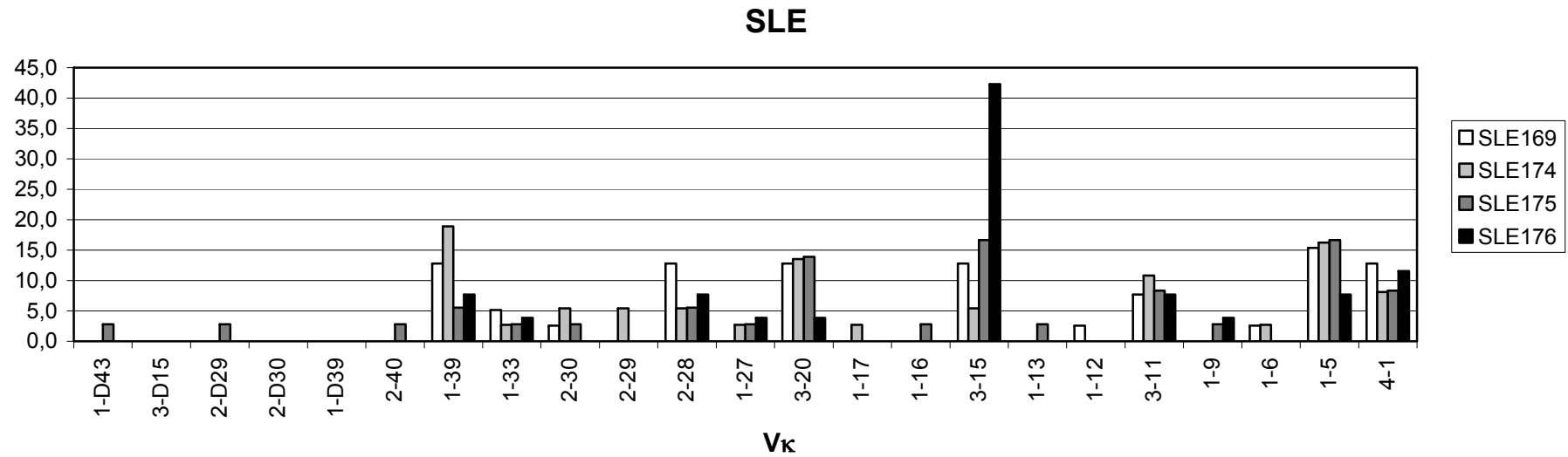


Figure 26: V_{κ} gene usage in IgG^{+} memory B cells of SLE patients 169, 174, 175, and 176. The frequency of individual V_{κ} genes is shown for each individual.

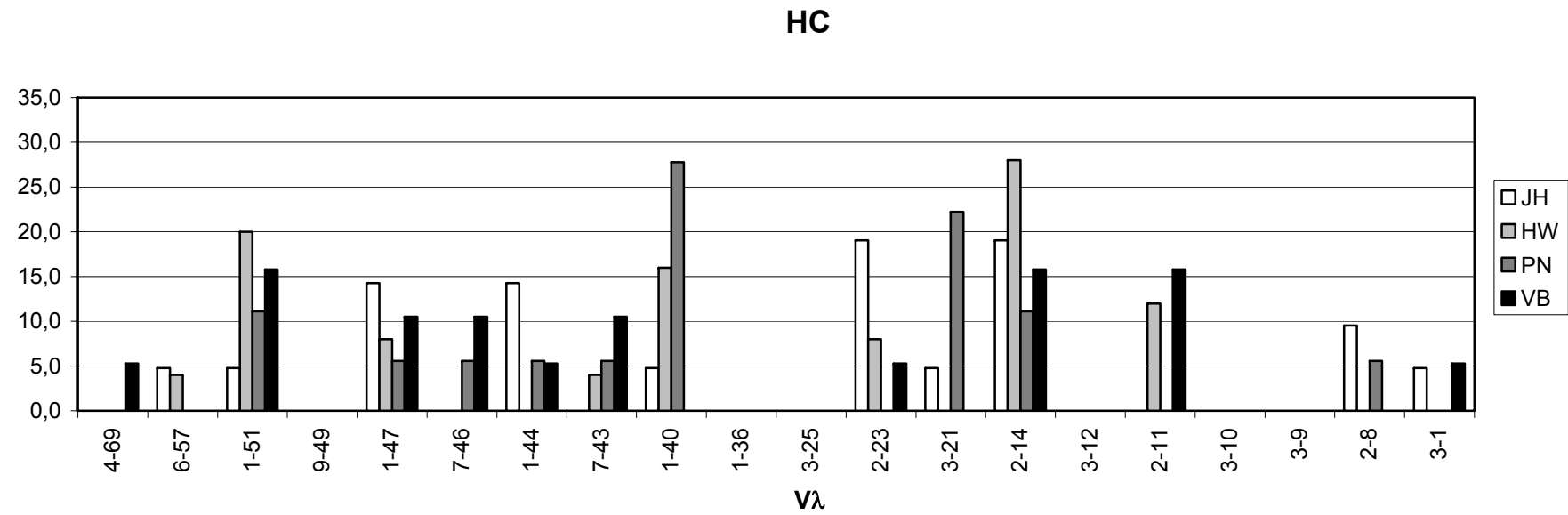


Figure 27: $V\lambda$ gene usage in IgG⁺ memory B cells of healthy donors JH, HW, PN, and VB. The frequency of individual $V\lambda$ genes is shown for each individual.

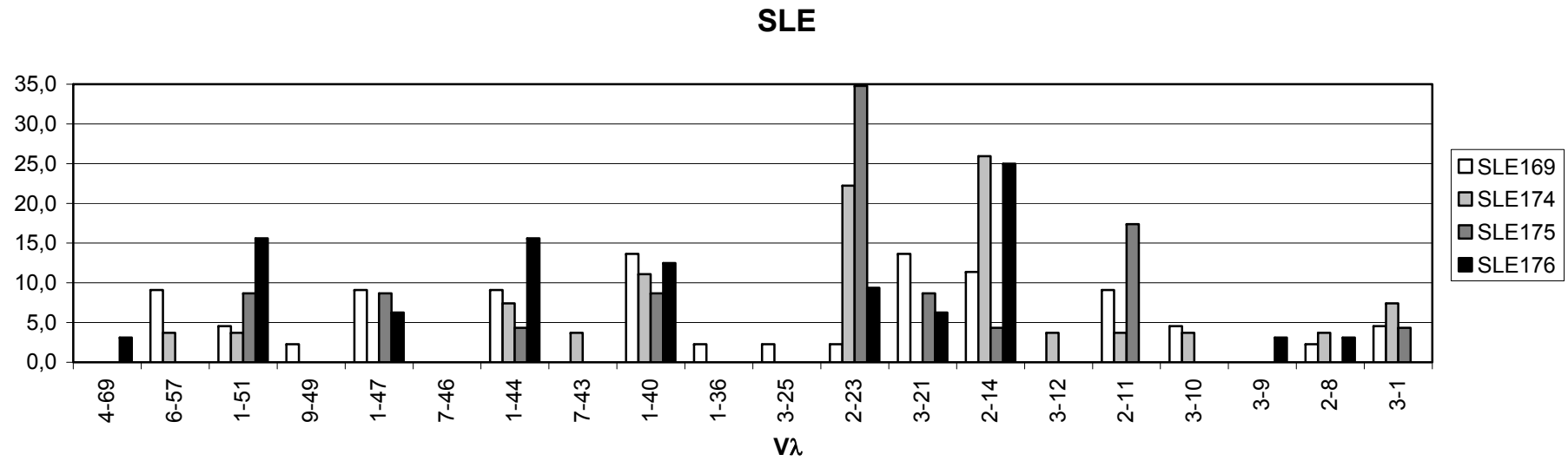


Figure 28: V λ gene usage in IgG⁺ memory B cells of SLE patients 169, 174, 175, and 176. The frequency of individual V λ genes is shown for each individual.

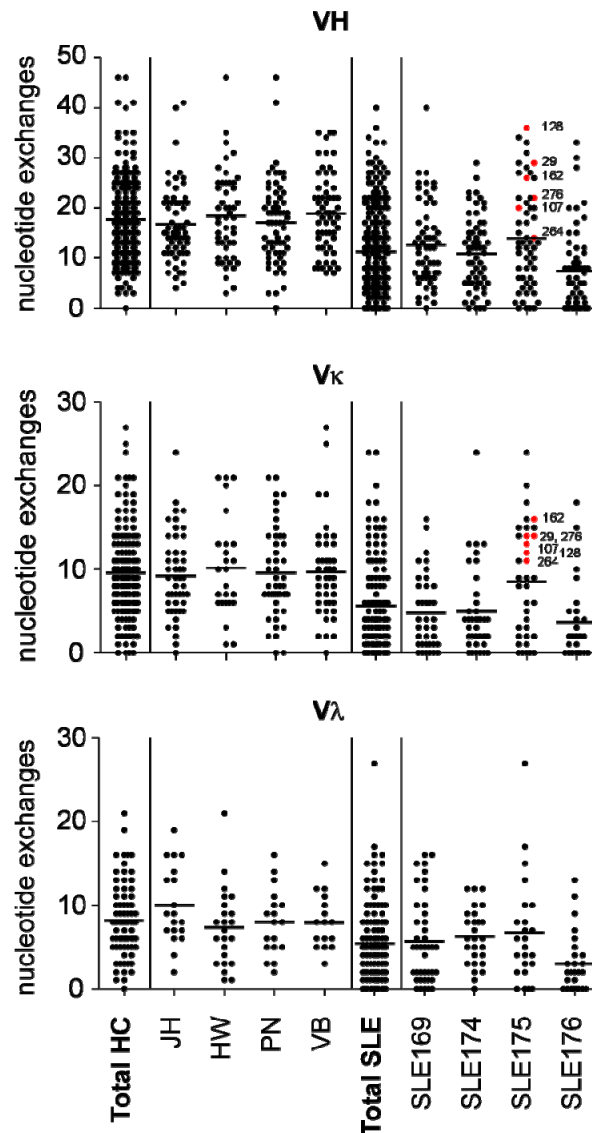


Figure 29: Number of somatic hypermutations in individual SLE patients and healthy controls. Dots depict individual VH, VK, and VL genes of healthy controls and SLE patients. Pooled data from healthy donors (Total HC) and SLE patients (Total SLE) is shown for comparison. Horizontal lines represent the average number of mutations in each individual. VH and VK genes of Ro and La specific antibodies from SLE175 are depicted in red and numbers indicate the respective antibody.

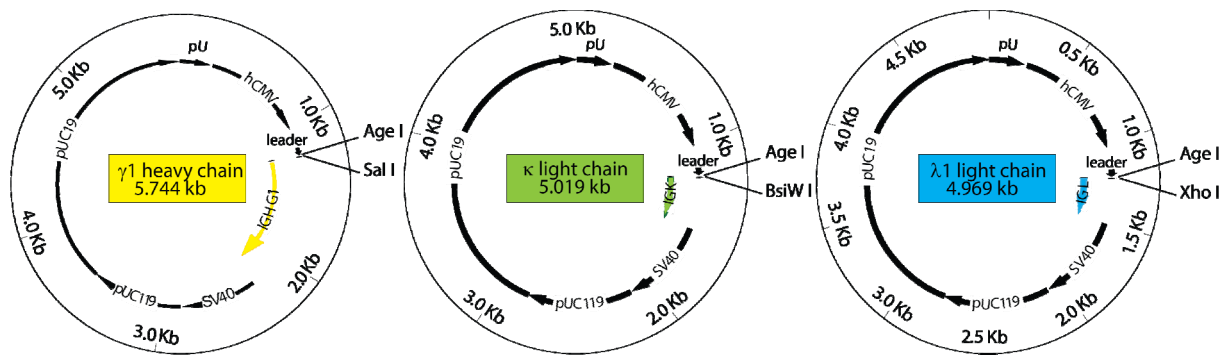


Figure 30: Vector maps of the applied eukaryotic expression vectors. Maps show the respective vectors for the cloning of IgH (yellow), Ig κ (green), and Ig λ (blue) chains. Restriction sites are indicated.

Supplemental tables

Table 5: Ig gene usage and features of antibodies cloned from single IgG⁺ memory B cells of patient SLE169

SUPPLEMENTAL INFORMATION

Ig	HEAVY					LIGHT					REACTIVITY							
gm/gp169	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	V _L	J _L	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2
gm169-3k	3-48	3-3	2	6	40	EDDFWVGSVYQTDYRFYPVDV	20	IgG1	3-20	1	0	QQYGGSSPWT	9	/	/	/	/	/
gm169-15	1-24	1-26	3	3	2	DRVEVGATEAFDI	13	IgG1	3-11	4	1	QQRSNWLLT	9	/	/	/	/	/
gm169-16k	1-8	/	/	6	8	RGSTVYYYYYGMVDV	14	IgG2	1-33	5	0	QQYDNLSS	8	/	/	/	/	/
gm169-20k	3-7	3-22	2	4	6	APPGSGSEYYFDY	13	IgG2	4-1	2	4	HQYYSPPYT	9	/	/	/	/	/
gm169-22k	3-23	2-2/15or3-22	2	4	22	DSCGSGTCRTRYHSDY	17	IgG1	3-15	2	6	QQYNRWPPHT	10	/	/	/	/	+
gm169-24k	4-4	3-3	2	5	14	GRDYDFWSGYSRQKEMYNWFD	22	IgG3	2-28	2	3	MQALQTPYT	9	+	+	+	+	+
gm169-28k	3-33	1-26	3	1	27	GNRLGATTFHH	11	IgG3	1-5	2	6	QQYKSLNT	8	+	+	+	+	+
gm169-31k	3-66	3-22	1	3	8	GNGYDDSSGYWRQWDAFDI	19	IgG1	1-39	1	1	QQSYSTPWT	9	/	/	/	/	/
gm169-34k#	4-4	2-15	2	3	15	ATEKYCRGSGSCYFHDAFDI	19	IgG1	1-39	1	8	QQSYSTPRT	9	/	/	/	/	/
gm169-38k	3-23	3-22	2	4	3	NIVNYDSSSGLPGY	14	IgG1	3-15	1	6	QQYNNWPRT	9	/	/	/	/	/
gm169-42	3-33	3-10	3	3	22	DANTVVRGVITPFDADF	18	IgG1	1-5	2	1	QQDRT	5	+	+	+	+	/
gm169-44	3-48	2-2	3	6	14	AKRDIVIPAADGYYYYYYMDV	23	IgG3	2-28	3	1	MQALQTPGT	9	/	/	/	/	+
gm169-46	4-39	5-24	2	3	24	QKDGYNPLSDAFYD	13	IgG1	1-39	4	0	QQSYSTPLT	9	/	/	/	/	+
gm169-57	3-23	6-6	2	5	13	GRDHSSSTWFDY	12	IgG1	1-5	4	1	QQYNIWLT	9	/	/	/	/	/
gm169-60	3-43	2-2	2	4	13	DLRFCSMNCPTGFDY	16	IgG1	1-33	4	9	QHYDNVLT	8	/	/	/	/	/
gm169-61	4-39	/	/	6	12	QEYTTSVYYYYYMDV	14	IgG1	3-20	4	8	QQYGGSSPLT	9	+	+	+	+	+
gm169-64k	3-7	6-19	2	2	4	DYGLSYSSVVFISINWYFD	19	IgG3	3-20	5	4	QQYGGSSPIT	9	/	/	/	/	+
gm169-68	4-34	6-6or6-13	3	4	1	EKTRSSSIARXKFFGAAGSGFLDY	25	IgG1	1-5	1	1	QQYNSYLTWT	10	+	+	+	+	+
gm169-70k	3-13	3-10	3	4	1	GVMMRGVITYYFDY	14	IgG1	1-39	5	0	QQSYSTLT	9	/	/	/	/	+
gm169-76k	3-11	/	/	6	2	DTPGRLVHHYYMDV	14	IgG1	2-28	2	6	VQALQTPYT	9	/	/	/	/	+
gm169-80k	4-39	1-26	2	5	14	DLGSSSFRF	10	IgG3	2-28	2	10	MQALQTPYT	9	+	+	+	+	+
gm169-86	4-4	2-21	2	4	6	RSYRGPFDY	9	IgG1	1-5	4	11	QQYNSYSSIT	10	+	+	+	+	+
gm169-94	3-30	3-10	2	4	27	GTGPGNYLTDY	11	IgG2	4-1	4	12	QQYYSAPLT	9	/	/	/	/	/
gm169-95	1-46	/	/	4	24	DKPEKRPQARTHYCDH	16	IgG1	1-39	4	15	QQSYTTRALT	10	/	/	/	/	/
gp169-8	1-24	3-22	2	3	0	VGDVYVYDGDALDI	12	IgG1	3-15	1	3	QQYNNWPWT	9	/	/	/	/	+
gp169-26k	3-33	6-19	2	4	25	DRDSSGLYFLDY	12	IgG1	4-1	5	8	QQYSSPT	8	/	/	/	/	/
gp169-29	4-4	2-2or3-10	3	5	6	DHLIVVPAAMSRVGGGELFPGWFD	27	IgG1	3-20	3	2	QQYGGSSPPT	9	/	/	/	/	/
gp169-53k	4-4	/	/	4	6	AQYVFDY	7	IgG1	4-1	3	16	QHYSSPST	9	/	/	/	/	/
gp169-64k	3-30	2-2	2	4	11	DWGGYCSSTSCSYFDY	16	IgG1	3-11	3	2	QQRFWHPRGIT	11	/	/	/	/	+
gp169-66	4-4	3-3	2	2	4	EGAYYDFWSGYNNLDWYFDL	20	IgG3	3-20	1	0	QQSGSSPT	8	/	/	/	+	+
gp169-81	5-51	3-3	2	3	17	GSTYDYSWNGPSDAFDI	17	IgG1	2-30	4	6	MQGTHWPLT	9	+	+	+	+	+
gp169-87	4-39	2-15	2	2	7	SHCSGDYCYSSWYFDL	16	IgG3	3-15	2	0	QQYNNWPRT	9	+	+	+	+	+
gp169-103k	4-39	3-9	1	5	25	VFRYDFDLWGWFDP	13	IgG1	4-1	5	11	QQYFSTPVT	9	+	+	+	+	+
gp169-108	1-2	/	/	4	7	EQVTRDFD	10	IgG1	2-28	2	3	MQALQIPRT	9	/	/	/	/	/
gp169-117#	4-59	3-3	2	3	6	VQQRSGPDDALDI	13	IgG1	1-5	2	6	QQYNSYSGYT	10	/	/	/	/	/
gp169-126	3-23	3-3	2	6	12	NNDFWSGGYYEV	11	IgG1	3-15	1	2	QQYNNWPWT	10	/	/	/	/	/
gp169-127k	1-69	/	/	4	9	DTGGGYFDY	9	IgG1	3-11	2	1	QQRSNWPPYT	10	+	+	+	+	/
gp169-129	5-51	4-23	2	2	5	PNYGGNNHYWYFDL	14	IgG1	1-12	4	3	QANSFPYPLT	11	/	/	/	/	/
gp169-144k	4-4	1-26	2	5	17	PGSGSYFGGYDNWFD	17	IgG2	1-6	5	9	LQDYNYPFT	9	/	/	/	/	+
gm/gp169	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	V _L	J _L	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2
gm169-3i									1-47	2	15	AAWDDSLSGVV	11	/	/	/	/	/
gm169-4	3-11	3-16or2-15	2	3	15	GGGTYSGAFL	11	IgG1	2-23	3	8	CSYAGVSTFW	11	/	/	/	/	+
gm169-8	4-34	2-15	1	6	16	GPRNSQLPLDYFYYYMDV	18	IgG2	1-47	2	14	AAWDDSLSGVV	11	+	+	+	+	+
gm169-11	1-2	/	/	6	9	ERIQVTSIARXKFFGYYGMDV	18	IgG3	1-44	1	5	AAWDDSLIGSV	11	/	/	/	+	+
gm169-16i#									2-11	1	ND	CAYAGDHKVV	10	/	/	/	/	/
gm169-20i									3-21	3	2	QVWDDSSSDHPL	11	/	/	/	/	/
gm169-22i									3-21	2	15	QVWDGIVV	8	/	/	/	/	/
gm169-24i									9-49	1	1	GADHSGSGNFVYV	13	+	+	+	+	+
gm169-28i									2-14	3	5	SSFTSSSTLAWV	12	+	+	+	+	+
gm169-31i									3-25	2	1	QSADSSGTSV	11	/	/	/	/	/
gm169-33#	4-34	6-19	2	5	18	DDRAWVLDS	10	IgG3	2-14	1	15	GCYTGRSSVY	10	/	/	/	/	/
gm169-34i									1-40	1	6	QSYDSSLSGYV	11	+	+	+	+	+
gm169-38i									3-21	3	1	QVWDDSSSDHOV	11	/	/	/	/	/
gm169-48	4-4	3-10or2-15	2	6	6	DVPTYCYGSGCYSYIDV	18	IgG1	1-44	1	13	ATWDDSLNGFV	11	/	/	/	/	/
gm169-64i									1-40	3	1	QSYDSSLGGSV	11	+	+	+	+	/
gm169-70i#									1-40	3	0	QSYDSSLSGWV	11	/	/	/	/	/
gm169-74	1-46	2-15	2	4	14	AGYCTGGSCFLVY	13	IgG1	1-40	1	4	QSYDSSLSGSV	11	/	/	/	/	/
gm169-76i									2-11	2	0	CSYAGSYTLV	10	/	/	/	/	/
gm169-80i									3-21	2	5	QVWDDSSSDHVV	12	/	/	/	/	/
gm169-83	3-74	/	/	4	8	DDDTFDY	7	IgG2	2-11	3	0	CSYAGSYTWV	10	/	/	/	/	/
gm169-85	3-48	1-26or5-12	2	5	12	GDSGIGP	7	IgG1	1-47	1	1	AAWDDSLSGPVY	12	/	/	/	/	/
gm169-88	3-30	2-15	2	4	9	SLSGGRCLRIDY	12	IgG1	3-1	2	7	QAWDTSTV	9	/	+	/	/	/
gp169-23#	1-58	6-13	3	6	ND	VNPPAAALMDV	11	IgG3	2-14	3	13	SSYTSGSTYVW	11	/	/	/	/	/
gp169-26i									1-44	3	6	AAWDDSLNGPV	11	/	/	/	/	/
gp169-32	4-59	/	/	6	9	DKAPKGMV	9	IgG3	3-21	3	5	QVWDDSSSDHPNVV	13	/	/	/	/	/
gp169-43	4-4	2-15	2	5	7	DWLNGYCSGGTCYSWFD	19	IgG3	1-40	2	0	QSYDSSLSVL	10	/	/	/	/	/
gp169-53i									2-14	3	10	CSYTNSTTLGV	11	/	/	/	/	/
gp169-64i									6-57	2	2	QSYDSSNVV	10	/	/	/	/	/
gp169-65	3-30	6-19	3	6	21	DERVAVAGMNNYYYYYGMV	19	IgG1	3-10	3	6	YSSDSSDKGV	10	/	/	/	/	/
gp169-71#	1-18	3-10	2	5	7	AVVYGGSGSYPIVLVDY	17	IgG1	1-51	3	1	GTWDDSSLSAGV	11	/	/	/	/	/
gp169-74	3-23	3-22	2	6	13	SKDYDIRGGYYSYPHYGMDV	21	IgG3	3-1	3	2	QAWDSSSVV	9	+	+	+	+	+
gp169-77	3-33	6-19	3	4	5	DRGSSGLYFYFDY	12	IgG1	6-57	3	2	QSYDSSNVV	9	/	/	/	/	/
gp169-82#	3-30	/	/	6	10	DSVTPEAYYYYYYGMV	16	IgG1	1-47	3	8	AAWNDTLSGPNVV	13	/	/	/	/	/
gp169-83	3-23	3-3	3	6	9	SPSRGATIFGVATPYCYMDV	21	IgG3	2-14	2	5	SSYSTNTPVV	11	+	+	+	+	+
gp169-85	3-43	1-26	3	6	25	DGGYTTGYFMDV	12	IgG1	1-36	3	16	AAWDDSLSAWV	11	/	/	/	/	/
gp169-86	3-30	6-19	3	6	23	DQRIAVAGMNNYYYYYGMV	19	IgG1	3-10	3	10	YSTDTSKGV	10	/	/	/	/	/
gp169-103i									6-57	3	2	QSYDSSNVV	9	/	/	/	/	+
gp169-113	1-69	3-22	2	3	12	EKAKITYYDSSGYPGGGALDI	21	IgG3	3-21	2	9	QVWESSDLVY	11	+	+	+	+	/
gp169-116	3-30	3-10	3	6	14	DDGSGDYYYAMDV	14	IgG1	1-40	3	9	QSYDSSLSGSWV	12	/	/	/	/	/
gp169-122	4-4	3-22	2	5	16	DRGYDDGSDYQYNWFD	18	IgG1	2-11	3	12	CSYAGTYFWV	11	/	/	/	/	/
gp169-127i									1-51	2	0	GTWDDSSLEGV	11	/	/	/	/	/
gp169-142	5-51	1-26	2	5	7	RSRESGSYSTWFD	14	IgG1	1-44	3	2	AAWDDSLNGSVV	12	/	/	/	/	/
gp169-144i#									2-8	3	5	SSYAGSFWV	9	/	/	/	/	/
gp169-148#	1-46	/	/	6	21	QGSAGEGLRSPAVVNSYGMV	22	IgG4	6-57	2	5	QSYDSSPVV	9	/	/	/	/	/

#, antibody was not expressed/tested; gm, IgG⁺ memory B cell; gp, IgG⁺ plasmablast; RF, DH gene reading frame; V-Mut., number of V gene mutations from FWR1 to CDR2, inclusively; SC, IgG isotype subclass; ss, ssDNA; ds, dsDNA; ins., insulin; +, reactive in ELISA/IFA; /, non-reactive in ELISA/IFA; ND, not determined

Table 6: Ig gene usage and features of antibodies cloned from single IgG⁺ memory B cells of patient SLE174

Ig	HEAVY						LIGHT						REACTIVITY					
gm174	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	Vk	Jk	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2
174-2	4-39	4-17	2	6	11	HQHADGDYAYYYYYGMDV	19	IgG1	2-28	1	1	MQALQMS	7	/	/	/	/	/
174-5	1-69	4-11/4	3	4	15	GLTIVTRFDY	10	IgG1	3-11	1	11	QQRSNWPPTWT	11	+	+	+	+	+
174-8	3-11	6-19	1	4	6	GGARGEQWLVLRY	13	IgG3	1-5	3	0	QQYDNPPS	8	/	/	/	/	/
174-9	1-2	3-22	2	5	12	GIYYDNFNGVGAHFDS	16	IgG1	4-1	4	4	QQYYGTPS	8	/	/	/	/	/
174-12#	1-46	/	/	6	5	GYRDFGYGLDV	11	IgG1	1-27	5	2	QKYNAPPAT	10	/	/	/	/	/
174-17#	3-23	6-13	1	5	4	GLQQLVRRGLDS	12	IgG1	2-29	3	2	MQSIQLPLFT	10	/	/	/	/	/
174-23	5-51	6-13	3	3	0	PAGIAAVDAFDI	12	IgG1	3-11	2	0	QQRSNWYT	8	/	/	/	/	/
174-24#	3-30	/	/	4	5	DGPNGGLCDY	10	IgG2	3-20	4	4	QQYGSSLLT	9	/	/	/	/	/
174-28	3-23	6-13	2	5	15	DVPYGTITWYGRGDS	14	IgG2	1-39	4	13	EQYTSAPLT	9	+	/	/	/	/
174-32	3-49	3-3	2	3	10	RNAYYYDDAFAI	12	IgG2	1-5	2	3	QQYNSYSYT	9	/	/	/	/	/
174-35	1-46	/	/	4	20	DGASNAWHIGY	11	IgG1	3-11	4	4	QHRINWRLT	9	/	/	/	/	/
174-55#	1-3	5-18/5	2	3	17	ERYAYGYAAFDI	12	IgG1	3-15	1	5	QQYNDWPPWT	10	/	/	/	/	/
174-60	5-51	6-19	3	5	19	RGDLAVAGTGFD	13	IgG3	1-39	1	2	QSYNTPWT	9	/	/	/	/	/
174-67	3-30	4-23	3	6	2	DLTTVDPSYYYYGMDV	17	IgG2	1-6	1	0	LQDYNYPWT	9	/	/	/	/	+
174-79#	3-30	/	/	4	15	DVGLGGDHWDYFDY	14	IgG1	1-39	5	5	QQSYSSPRIT	10	/	/	/	/	/
174-84	4-34	/	/	6	3	LESMLGLGYYYMDV	16	IgG1	1-39	1	2	QSYSTPWT	9	/	/	/	/	/
174-87	3-48	6-13	2	4	1	VGRTSPLYSSWYGFY	17	IgG1	3-20	3	0	QQYGSSPFT	9	/	/	/	/	+
174-91#	4-31	/	/	4	0	DPPRATPFDL	10	IgG1	1-17	2	4	LQHNSSPYT	9	/	/	/	/	/
174-117#	4-34	6-13	3	4	8	GIAAFGY	7	IgG1	1-39	2	4	QSYSTPRT	9	/	/	/	/	/
174-119	3-48	2-2	2	4	7	GGYCTSTSCHEPLFGVMTIPRYFDY	26	IgG1	3-20	5	2	QQYGSSPT	8	/	/	/	/	/
174-120#	1-24	/	/	3	5	VTEGGRFADFID	12	IgG1	2-30	2	0	MQGTHWPMYT	10	/	/	/	/	/
174-129	3-30	2-15	2	5	17	GCSSSGNCFIDY	13	IgG2	4-1	4	13	QQYNSPPT	9	/	/	/	/	/
174-130	4-31	5-12	2	4	1	EGVYSAFPSAY	11	IgG1	3-11	3	1	QQRSNWPSFT	10	+	+	+	+	+
174-133	1-69	6-13	3	6	21	GIVAADYYGMDV	12	IgG4	2-28	2	4	MQSLQTSFT	9	/	/	/	/	/
174-140	4-59	/	/	6	29	EDAYGMDV	8	IgG1	1-39	1	24	QQNDRAPRT	9	/	/	/	/	/
174-146#	3-72	3-22	2	4	9	LAGDGSYYIY	12	IgG2	3-20	4	12	QHFRGSPALT	10	/	/	/	/	/
174-148#	5-51	3-22	3	4	7	QGIVVPATWEGGFY	15	IgG1	1-33	2	13	QQYGNLPCG	9	/	/	/	/	/
174-154	4-34	3-9	2	3	0	SAAYYDILTGYADAFDI	18	IgG2	1-5	2	0	QQYNSYSPYT	10	+	+	+	+	+
174-156	4-39	2-2	2	4	14	RRORGPDCSSTSCYFDY	17	IgG1	1-5	1	4	QHYDDFA	7	/	/	/	/	/
174-157#	3-53	6-19	2	4	6	VGYSWGYYRD	10	IgG1	3-15	1	4	QQYNNWPPGT	10	/	/	/	/	/
174-169#	3-23	3-22	2	6	15	DRSETSGSYSPYYLMDV	21	IgG2	1-5	1	11	QQYSTYSRT	9	/	/	/	/	/
174-176	3-23	/	/	4	0	DGVRDSPSTFDY	12	IgG2	4-1	3	1	QQYSTPPS	9	/	/	/	/	+
174-177	5-51	/	/	6	5	PSAMDYYYAMDV	12	IgG1	3-20	1	2	QQYGSSPWT	9	/	/	/	/	/
174-179#	4-39	5-12	2	6	9	RYSGPVGFGLDV	12	IgG1	1-39	5	7	QQSYSSPRIT	10	/	/	/	/	/
174-184#	4-39	3-22	2	4	8	QDYDSSGYSYAFDY	14	IgG1	1-5	2	3	QQYNSYSPYT	10	/	/	/	/	/
174-194#	4-31	/	/	4	11	GLTSFDY	7	IgG1	2-29	4	6	MQSIQLPLT	9	/	/	/	/	/
174-196	3-7	/	/	3	23	DPEFSAFDI	9	IgG1	2-30	1	9	MQATHWPS	8	/	/	/	/	/
gm174	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	Vk	Jk	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2
174-1	1-69	3-22	2	6	9	GDYASSGYHHGMDV	15	IgG1	3-10	2	3	YSTDSSGNHGV	11	/	/	/	/	/
174-16	4-28	/	/	6	5	RVGYLDV	7	IgG1	2-23	3	12	CSYAGSGTWV	10	/	/	/	/	/
174-18#	5-51	2-15	3	5	4	GEVVAHNCDFP	11	IgG1	2-14	3	2	SSYTSSLTGV	10	/	/	/	/	/
174-29	3-15	3-22	2	3	13	EPLVEMYDFDI	11	IgG2	3-1	1	6	QAWDNSTARV	10	/	+	/	/	/
174-33	3-21	2-2	2	4	11	VRLGYCSNIRCAENKNFDF	19	IgG1	1-40	3	6	QSYDNKLRRV	10	+	+	+	+	+
174-44	3-33	3-16	2	4	22	GVWGNYPDPY	10	IgG1	2-23	3	12	CSYAGSRTWV	10	/	+	+	/	/
174-45	3-53	6-19	3	4	16	VLVQWLVPGNYFDY	14	IgG2	2-11	1	8	CSYAGTYTYV	10	+	+	/	/	/
174-54	4-59	2-15	2	2	13	RGTLSGCTGAHCATDWFYDL	20	IgG2	3-12	6	9	QAWDSSSDHPGV	12	+	+	+	+	+
174-59	3-33	/	/	5	1	GEAWGTNWFD	11	IgG3	2-23	2	1	CSYAGSSTPVV	11	/	/	/	/	/
174-63	3-74	/	/	4	17	GHQPQYTKHFDY	12	IgG1	7-43	3	8	LLYFGGFVW	9	/	/	/	/	/
174-68	3-7	2-8	2	4	12	VRRDCTNGVCTYYFDY	16	IgG2	2-23	3	5	CSYAGSRNYWV	11	/	/	/	/	/
174-71	1-46	3-22	3	4	6	APGPVLITRPYYFDY	15	IgG2	2-23	3	8	CSYENSINPNWV	12	+	+	+	+	+
174-77	3-15	3-9	1	4	6	DGNFDY	6	IgG2	2-8	3	4	SSYAGSNNWVW	11	/	/	/	/	/
174-82	5-51	4-23	2	4	19	THDGNFFWDY	10	IgG2	2-14	1	8	SSYTRTNTVTYV	12	/	/	/	/	+
174-114	1-18	3-10/3	3	5	22	DRVVRGPITGRNWLD	16	IgG3	2-14	3	12	TSYTTSSTWV	10	+	/	+	/	+
174-115#	4-39	3-3	2	5	12	SKNDNDFWSAFATGWFD	18	IgG1	2-14	2	3	SSDTSSTLSG	11	/	/	/	/	/
174-118	4-59	3-22/9/3	2	3	14	VSAVEDGYWLVGAFDI	16	IgG1	1-44	2	5	AAWDDSLNGHVV	12	/	/	/	/	/
174-120#									2-14	3	5	SSYTSSSTWV	10	/	/	/	/	/
174-125#	7-81	6-13	2	4	26	MMYSSSWNYFDS	12	IgG1	1-40	3	0	QSYDSSLSGSV	11	/	/	/	/	/
174-127	3-33	5-24	3	4	12	SRMTFFFLDY	11	IgG1	6-57	3	3	QSYDSFNQV	9	/	+	/	/	+
174-131	4-28	/	/	5	3	VKPVATFDY	9	IgG1	1-51	2	4	GTWDNLSLACQ	11	/	/	/	/	/
174-151	3-53	6-13	3	4	5	GPFSAGNHFDY	12	IgG1	3-1	2	2	QAWDNNTPV	9	/	/	/	/	/
174-162#	5-51	4-17	2	6	21	RRYGDHEGGLDV	12	IgG1	2-14	2	9	SSYTSDSTVI	10	/	/	/	/	/
174-165	3-53	1-26	2	3	13	GSTLVGDKGDAFDI	14	IgG1	2-14	2	6	SSYTSTNNLL	10	/	/	/	/	/
174-174	4-39	4-17	2	5	13	REDYGDYGFDS	12	IgG1	2-23	3	10	CSYAGRSIM	9	/	/	/	/	+
174-180	5-51	4-17	2	4	12	HDEYDGF	7	IgG1	1-40	3	7	QSYDTSLSASL	11	/	/	/	/	+
174-183#	3-11	/	/	4	23	GPVITVELDY	11	IgG1	1-44	2	10	STWDDSLV	9	/	/	/	/	/

#, antibody was not expressed/tested; gm, IgG⁺ memory B cell; RF, DH gene reading frame; V-Mut., number of V gene mutations from FWR1 to CDR2, inclusively; SC, IgG isotype subclass; ss, ssDNA; ds, dsDNA; ins., insulin; +, reactive in ELISA/IFA; /, non-reactive in ELISA/IFA; ND, not determined

Table 7: Ig gene usage and features of antibodies cloned from single IgG⁺ memory B cells of patient SLE175

Ig	HEAVY										LIGHT										REACTIVITY				
gm175	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	V _κ	J _κ	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2							
175-19	3-23	3-22	2	5	15	QREYHYDSSDYDYEDS	16	IgG1	1-5	4	15	QQYLGVT	7	/	/	/	/	/							
175-29	5-51	7-27	3	4	29	LPLTGGACDY	10	IgG1	3-15	1	14	QQYNKWWT	8	+	+	+	+	+							
175-39#	3-43	2-21	2	4	17	PGGFLMVGFND	13	IgG1	3-15	4	18	QQYNKGLT	8												
175-62	4-61	3-10	2	5	17	DPGYGSGSYQIWFDP	15	IgG1	1-13	4	15	QQYYTLLT	9	/	/	/	/	/							
175-77	3-23	6-19	3	4	13	PASVAGQGGVDY	12	IgG1	1-5	1	9	QQYNTYFQWT	10	+	+	+	+	+							
175-79	3-73	1-26	3	5	27	TSYVVGAPT	9	IgG1	2-28	4	8	MQALQTPLT	9	/	+	+	+	/							
175-86#	3-23	2-21	3	4	14	GGVVVTAITFFDY	13	IgG1	3-15	1	0	QQYNNWPLWT	10												
175-90	4-4	3-22	2	3	13	SLRDDYYDSSGYKDAFDI	19	IgG1	1-5	1	2	QQYDTSYWT	9	/	/	/	/	/							
175-95#	4-34	6-19	3	2	19	ATRAVAGIIGRVSYFDF	17	IgG3	1-39	5	20	QQSYTMT	8												
175-107	3-7	1-20/7	3	2	20	TARIAGTGGGYFDV	14	IgG1	1-5	2	13	QQYKSYT	7	/	/	+	/	+							
175-108	1-69	2-15	2	4	5	SGGGYCSGGSCYSADY	16	IgG1	3-11	2	0	QQRSNWPPHT	10	/	/	+	/	+							
175-113#	3-33	3-22	2	4	6	QGWPNYYDSSGYPSVDY	18	IgG3	1-33	4	2	QQYDNLPPWLT	11												
175-116	4-34	/	/	4	8	KGWELLYFDY	10	IgG1	4-1	2	6	QQYYSTPYT	9	/	/	/	/	/							
175-128	3-23	3-10	3	4	36	DGRRGFLRLVDYLD	15	IgG1	2-28	1	12	MQALQTPPT	9	/	/	/	/	+							
175-140	4-59	6-19	3	5	18	DRAVAAAMYNWVDL	13	IgG1	1D-43	4	24	HQYYGTPLT	9	+	+	+	+	+							
175-150#	1-18	3-10	3	4	3	SELRSTMVRGVIPRGYFDY	20	IgG1	3-15	1	2	QQYNNWPEP	9												
175-153	3-7	/	/	4	28	EKSGTYFDY	10	IgG1	3-11	4	9	QQRINPLT	8	/	/	/	/	/							
175-157	3-23	/	/	4	27	EKVVVPSALYDY	14	IgG1	1-9	2	15	LQLNTNPYT	9	/	/	/	/	/							
175-162	3-33	2-2	3	4	26	QGWGNWRENAVARHYFDF	19	IgG1	1-5	1	16	QQYNSSSPT	9	/	/	+	/	+							
175-217	1-8	6-19	2	4	8	GGGNSGWPTPYFND	15	IgG1	4-1	4	0	QQYKAPSLT	10	/	/	/	/	/							
175-229#	3-30	/	/	4	29	GANTVRWDVPVHFDN	14	IgG1	3-20	5	16	QQYGRSPIT	9												
175-233	3-15	3-9	2	5	22	VQRGDILTEYCNS	13	IgG1	2-30	1	11	MQGTHWTWT	9	/	/	/	/	/							
175-240	1-2	6-13	3	6	22	DSNIASPGIGTMDV	14	IgG3	1-39	4	14	QQTYSNPLT	9	/	/	+	+	+							
175-241	4-31	6-13	2	4	6	LLTGSSSWYFDDY	13	IgG1	1-27	1	1	QKYNAPWT	9	/	/	/	/	+							
175-250	1-2	1-26	3	4	22	DRAIVGAANDFY	13	IgG1	3-20	1	8	QCGSSPT	8	/	/	/	/	/							
175-258#	3-23	3-22	2	4	11	ERHYDSSGGIDY	13	IgG1	1-16	4	6	QQYKSYPLT	9												
175-260#	3-30	3-22	2	4	20	DGLYFDRSGFFHFHDH	16	IgG1	4-1	2	9	QQYASAPPT	9												
175-263#	1-8	/	/	6	1	LQFEYSASYGMDV	14	IgG1	3-15	4	3	QQYNNWPLT	9												
175-264	3-33	6-19	2	1	14	DGGAYSSGWYPQH	13	IgG1	3-15	2	11	QQYNVRPYT	9	+	+	+	/	+							
175-265	3-23	/	/	4	13	RKTYYFDY	8	IgG1	3-11	5	5	QQRSDWPIT	9	/	/	/	/	+							
175-266	3-15	6-19	2	4	1	VDSSVWYGHPIFDY	14	IgG3	3-20	1	4	QLYGISLWT	9	/	/	/	/	/							
175-269	1-3	3-22	2	4	0	DGGPYDSSGFGGADYFDY	19	IgG1	3-20	1	0	QQYSSSPGT	9	/	/	/	/	/							
175-276	1-24	3-10	3	5	22	PYFSTSGSLGDLFDP	15	IgG1	2-40	1	14	KQRLDGVT	8	/	/	/	/	+							
175-277	3-21	6-19/13	3	6	3	PVTPGIALYGVDDV	13	IgG3	1-5	2	3	QQYNSDST	8	/	/	/	/	/							
175-285#	3-9	/	/	4	4	AAFGRLLVSGGLFDY	15	IgG1	2D-29	2	1	MQSIQLGYT	9												
175-292#	1-69	4-17	3	3	0	RGGRGDYPYAFDI	13	IgG1	3-20	2	0	QQYSSSPYT	9												
gm175	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	V _κ	J _κ	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2							
175-23	4-39	3-22	2	4	21	HVYHDSSTTYFESHYFDS	18	IgG1	2-14	3	15	GSYTGNSSLYVW	12	/	/	/	+	/	+						
175-42	3-74	/	/	6	9	ELPGGYGMDV	10	IgG1	2-23	3	4	CSYAGSLTLV	10	/	/	/	/	/							
175-80	4-34	3-10	2	3	0	GDYYSRHAFFDI	12	IgG1	2-11	3	0	CSYAGSYGWW	10	/	/	/	/	/							
175-82#	1-3	4-17/11/4	2	5	4	GGRSSYRDDYGDYVWFDP	19	IgG1	1-40	1	3	QSYDSSLGYY	11												
175-101#	4-39	6-19	3	6	13	TFPDYIAVAGPPRYGYMDV	20	IgG3	1-47	1	0	AAWDDSLSAFYV	12												
175-114#	1-3	/	/	4	12	YISPEDPFDDY	10	IgG1	2-23	1	6	CSYAGSSLYV	10												
175-142#	4-31	/	/	6	26	AGALRTVPPYYAMDV	15	IgG1	2-23	3	17	CSNAGGNIFLV	11												
175-146#	4-34	3-10	1	5	20	GGGEGSFD	9	IgG1	1-51	2	7	GTWDDSSLSAVV	11												
175-160	3-23	/	/	4	8	DQAGSRDLY	9	IgG1	2-11	1	2	CSYAGTYTYV	10	/	/	/	/	/							
175-165	3-23	5-18/5	1	3	10	DPFRWMQLWTTDAFDI	16	IgG1	1-44	1	2	ATWDDSLNGRV	11	+	/	/	/	/							
175-169	1-8	5-18/5	2	4	1	AHFQRGYKDY	10	IgG1	1-47	2	0	AAWDDSLSGVW	11	/	/	/	/	+							
175-215	4-28	3-10	2	4	12	NYGSLYFDDY	11	IgG2	2-23	2	10	CSCAGSGTVV	10	/	/	/	/	+							
175-236#	3-49	6-13	1	3	6	EQQLTSAHDVFDI	13	IgG1	3-21	2	4	QVWDDSSSDHVV	11												
175-238	1-2	4-17	3	6	10	DTVTTTYHYGLDL	13	IgG3	1-40	2	8	QSYDSSLRTVV	11	/	/	/	/	/							
175-242	3-11	/	/	4	5	AQGYFDY	7	IgG1	2-11	2	4	CSYAGSYTYV	10	/	/	/	/	/							
175-248#	7-81	3-22	2	3	33	RGLHPYDYDTSYSAFDI	18	IgG3	3-21	3	5	QVWDDISSDHGV	11												
175-249#	3-33	3-10	3	4/5	3	VKGYSRMTLVRGVINDY	17	IgG3	2-23	3	7	CSYAGSGTLW	10												
175-270#	4-34	5-18/5	3	5	11	VDTAVVLEY	9	IgG3	1-51	3	6	GTWDDSNLSAGV	11												
175-280	3-48	4-17	2	6	12	EGYGDYLRYYAMDV	14	IgG1	2-23	1	10	FSYAGGSFTYV	11	/	/	/	/	/							
175-282	3-74	1-26	2	4	8	LGGGSYPYFLDY	12	IgG1	3-1	2	8	QAWDDSDTVV	9	/	/	/	/	/							
175-286	4-61	/	/	3	34	VENDSFDDV	8	IgG2	2-23	3	27	CSYAGNKWV	9	/	/	/	/	/							
175-289	3-11	/	/	6	0	VMGGMDV	7	IgG1	2-11	3	0	CSYAGSFWV	9	/	/	/	/	/							
175-295	3-9	6-13	2	4	3	GRLRSSTWGYFDY	13	IgG1	2-23	3	3	CSYAGSSTWV	10	/	/	/	/	+							

#, antibody was not expressed/tested; gm, IgG⁺ memory B cell; RF, DH gene reading frame; V-Mut., number of V gene mutations from FWR1 to CDR2, inclusively; SC, IgG isotype subclass; ss, ssDNA; ds, dsDNA; ins., insulin; +, reactive in ELISA/IFA; /, non-reactive in ELISA/IFA; ND, not determined

Table 8: Ig gene usage and features of antibodies cloned from single IgG⁺ memory B cells of patient SLE176

Ig	HEAVY										LIGHT										REACTIVITY				
gm176	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	Vκ	Jκ	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2							
176-10	3-48	2-15	3	4	5	VAATGGSRRFFDY	12	IgG1	1-33	5	2	QQYDNLDPDT	9	/	/	/	/	/							
176-62	3-23	2-2	2	5	8	EVEYCSSTSCNNRGRWFPD	21	IgG2	3-15	2	0	QQYNNWRT	8	/	/	/	/	/							
176-66	4-31	3-22	2	4	28	ESRPDRSGYYAFEF	14	IgG1	1-5	1	2	QQYNSYSRGT	10	+	+	+	+	/							
176-91#	3-21	6-13	2	3	ND	DRASHGSWTENAFV	15	IgG1	1-5	4	18	QQYESYPLT	9												
176-172#	3-21	3-9	1	4	1	AKLQYFDWLLSGPIDY	16	IgG1	3-15	1	0	QQYNSYSWT	9												
176-214	1-69	3-22	2	4	0	SFPIRSYDSSGHFDY	15	IgG1	3-15	1	0	QQYNNWPPGT	10	+	+	+	/	/							
176-236#	4-59	5-24	2	4	4	LDGYMPEGNYYFDY	14	IgG1	3-15	5	2	QQFNSYPIT	9												
176-256	1-18	1-1/20	2	4	21	GSLNWKESGVY	12	IgG2	3-15	5	4	QQYNNWPPIT	10	/	/	/	/	+							
176-267	3-23	3-22	2	3	8	DRRPSSDYDDAFDV	15	IgG1	3-15	1	5	QQYDSYPGT	9	/	/	/	/	/							
176-270	4-34	3-10or7-27	2/3	4	2	VSGWPGSYITGSDNY	16	IgG1	3-15	2	0	QQYNSYPYT	9	+	/	+	+	+							
176-301	3-33	/	/	6	7	EPRARWYGMVDV	12	IgG2	2-28	2	4	MQALQTPHT	9	+	+	/	/	/							
176-337	3-33	6-19	2	4	1	GEVEALKYSSGWTGALWDY	19	IgG3	2-28	1	0	MQALQTLWT	9	/	/	/	/	+							
176-435	1-69	2-21	3	6	1	ASDIVVTAYYYYGMDV	18	IgG3	3-15	2	1	QQYNNWPPIT	10	+	/	/	/	+							
176-514	1-69	4-23	3	6	3	DGGAGKSDVTPRGYYYMDV	21	IgG1	1-39	3	15	RQSYSTPVT	9	+	/	+	/	/							
176-534	3-21	3-10	2	4	0	TAYYSGSGSYFGLYYFDY	18	IgG3	3-15	1	2	QQYNNWPPG	9	+	/	+	/	/							
176-548	3-21	3-22	2	3	15	RYYYDSSSDFADI	14	IgG2	1-27	3	9	KYDGPAPT	9	/	/	/	/	+							
176-585	4-59	3-3	2	4	18	GISPYSRFWSGYKVHYFDY	19	IgG1	3-20	1	10	QQYGTSPGT	9	+	+	+	+	/							
176-592#	4-31	3-3	3	4	2	VAIFGVVIDY	11	IgG3	3-11	4	0	QQRSNWPLT	10												
176-611	3-9	2-2	1	4	1	DKYGNQLLSGPFDY	14	IgG3	4-1	2	3	QQYYSTPPT	10	+	+	+	/	+							
176-618	1-58	3-22	2	6	2	DDRSADV	7	IgG1	3-11	5	1	QQRSNWPPGIT	11	/	/	/	/	/							
176-621	4-34	2-2	3	6	17	EIVPPASYYYIDV	14	IgG1	1-9	2	6	QQLNTYPRT	9	/	/	/	/	/							
176-632	4-59	5-12	2	2	0	SRGYSGYDNLWYFDL	15	IgG3	4-1	3	0	QQYYSTPFT	9	/	/	/	/	/							
176-641	3-23	6-19	2	4	8	VPGYSSGWFSYD	12	IgG2	3-15	1	3	QQYNNWPLA	9	/	/	/	/	/							
176-650#	3-9	1-26	2	5	6	DSSVYSGRYFGIDS	14	IgG3	4-1	4	5	QQYYSTPRLT	10	/	/	/	/	/							
176-655	4-39	3-9	2	3	0	LSYDILTDPAFDI	14	IgG3	3-15	2	0	QQYNNWPPMYT	11	/	/	/	/	/							
176-694	3-48	/	/	4	8	DPTRLRLRGYFDY	13	IgG3	1-39	1	2	QQSYSTPRT	9	/	/	/	/	+							
gm176	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	Vκ	Jκ	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2							
176-29	3-48	2-15	3	4	6	VAATGGSRRFFDY	12	IgG1	2-14	3	0	SSYTSSSTLV	10	/	/	/	/	/							
176-130#	4-4	5-12	2	4	20	GYSSFSPDY	9	IgG1	1-44	3	ND	ATWDDSLNGWV	11												
176-223	4-34	2-2	3	5	0	DPIVPAVGWGFDP	14	IgG3	1-40	2	0	QSYDSSLAHV	12	/	/	/	/	/							
176-226	1-46	6-19	2	4	11	SYTSGWLYYLD	12	IgG1	1-51	3	4	GTWDNLSAGV	11	+	+	/	+	/							
176-229	3-21	5-18	2	6	8	EGYSYGEHQGAMDV	14	IgG2	1-44	3	3	AAWDDSLNGRV	11	/	/	/	/	/							
176-241	3-9	2-2/8	2	4	5	DMGHSRYTPRIDY	13	IgG2	2-14	2	0	SSYTSSSTHVV	11	/	/	/	/	/							
176-253#	4-39	4-4/11	3	6	15	VDPTVTGLYYGMDV	15	IgG1	1-40	2	3	QSYDSSLGYYV	12												
176-272	4-39	5-5/18	2	4	11	HRGYSYGDY	9	IgG1	3-9	3	1	QVWDSTSWV	9	+	+	+	+	+							
176-275	1-8	2-2	2	4	1	GRRGYCNSSTCLYYFDY	17	IgG3	1-51	2	0	GTWDSSLSAVV	11	/	/	/	/	/							
176-287#	1-2	6-13	3	5	10	DRPVDVTNPSGIWFPD	16	IgG1	1-40	2	3	QSYDSSLSDVL	11												
176-290#	4-34	6-13	3	5	0	GASRDSTAKIAAAVDY	16	IgG1	2-14	3	2	SSYTSSSTRV	10												
176-310	3-7	3-10	1	4	12	CGELLTSVPFDF	12	IgG1	1-47	3	7	ASWDDSLTSWV	11	/	/	/	/	/							
176-318#	5-51	5-5/18or3-10	2	4	20	SVYTYGPVDY	10	IgG1	1-40	3	13	QSYDSSLAKWV	12												
176-323	1-69	3-22	2	3	0	GYDSSGYQIYAFDI	14	IgG2	2-14	1	2	SSYTSSSTLYV	11	/	/	/	/	/							
176-365	4-31	5-24	3	3	9	GGVEMDGDFAFI	12	IgG1	3-21	2	2	QVWDSSSHRVI	11	/	/	/	/	/							
176-392	4-4	/	/	4	9	GKAFRYFDY	9	IgG1	2-14	3	2	SSYTSSNTLD	10	/	/	+	/	+							
176-393#	4-61	3-10	2	5	30	ADDHGAGTHFYPGWFPD	17	IgG1	2-14	2	ND	SSCMGLNTPV	11												
176-395	4-59	4-4/11	3	6	8	APTVTITYSYYYMDV	14	IgG1	3-21	2	4	QVWDSSSDHLV	12	/	/	/	/	/							
176-428#	1-18	3-3	3	4	0	DPGITIFGVVIVPLYFDY	19	IgG1	2-14	1	5	SSYTSSNTYV	10												
176-447#	4-59	2-2	3	6	3	ADIVAETDYYYYYMDV	16	IgG1	2-14	2	ND	SSYTSSSTPYV	12												
176-508#	4-4	6-13	2	6	5	DSSSWPAVHMDV	12	IgG1	2-23	2	ND	CSYVGSNVV	9												
176-523	3-30	/	/	6	12	GGSPVYYYYMDV	12	IgG1	1-51	1	2	GTWDNNLSAGV	11	/	/	/	/	/							
176-535	3-11	6-13	1	3	0	AVTGLVQQLVFTTGARIDAFDI	22	IgG3	2-23	2	0	CSYAGSSTSV	11	/	/	/	/	/							
176-536	1-2	4-4/11/17	2	4	0	GVESSDY	7	IgG1	2-23	1	0	CSYAGSSTYV	10	/	/	/	/	/							
176-540	3-30	3-22	2	6	5	SPPPPYYDSSGYRVDYMDV	21	IgG2	1-51	2	0	GTWDSSLSARV	11	/	/	/	/	/							
176-558	3-48	6-19	3	4	8	GIALANSDFDH	11	IgG1	1-47	3	9	AAWDASLRGWV	11	/	/	/	/	/							
176-614	4-39	5-5/18	3	4	0	RGWDTAMAQGYFDY	14	IgG1	1-44	3	0	AAWDDSLNGWV	11	/	/	/	/	/							
176-616	3-9	3-22	2	3	2	DLYYDSSDYDAFI	14	IgG1	1-44	2	1	AAWDDSLNGLV	11	/	/	/	/	/							
176-620	3-9	2-8	2	3	13	PDCTNGVCSYGAFDI	15	IgG3	2-8	2	6	SSYAGNNIV	10	/	/	/	/	/							
176-627	3-30	2-2	2	4	33	GVFCPGTNCYVWGLPTN	17	IgG1	1-44	3	11	ASWDDSMRSPV	11	/	/	/	/	/							
176-650#									4-69	2	ND	QVWGTGIYV	10												
176-662#	3-30	/	/	4	ND	DHAQSWRSNYFDY	13	IgG3	1-51	2	0	GTWDSSLSAQV	11												

#, antibody was not expressed/tested; gm, IgG⁺ memory B cell; RF, DH gene reading frame; V-Mut., number of V gene mutations from FWR1 to CDR2, inclusively; SC, IgG isotype subclass; ss, ssDNA; ds, dsDNA; ins., insulin; +, reactive in ELISA/IFA; /, non-reactive in ELISA/IFA; ND, not determined

Table 9: Ig gene usage and features of antibodies cloned from single IgG⁺ memory B cells of healthy control JH

Ig	HEAVY										LIGHT										REACTIVITY					
gmJH	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	V _k	J _k	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2								
JH1	1-18	5-12	3	4	12	DSGATMVPFY	10	ND	3-20	4	10	QQYGSPLT	9	/	/	/	/	/								
JH5	3-30	3-3	2	4	12	DLESYDFWWSGLNDY	14	IgG1	3-20	1	7	QQYGRSQKT	9	/	/	/	/	/								
JH17	4-34	5-12	3	4	15	WSVDIVTTTRGTCKNYFDD	19	IgG1	3-11	1	3	QQRSDWPKT	9	/	/	/	/	/								
JH19#	4-59	5-12	2	4	6	MMPSGYVRV	9	IgG2	3-20	1	8	QQYGSTWT	8													
JH20	3-33	3-9	1	6	12	DQPKGGSRYFDWLSADGMDV	20	IgG1	3-15	4	7	QQYNNWPPLT	10	+	+	+	+	/								
JH22#	1-24	6-6	3	6	15	GGIAARPLYFYHALDV	16	IgG1	1-39	5	12	QQYTSAPLS	9													
JH23#	1-18	3-10	3	6	13	DSLITLVREELLYYHGMVDV	20	IgG1	3-20	4	8	QQYKSPRLT	10													
JH26#	5-51	4-23	2	4	14	SSHGGNSRSDY	11	IgG1	3-20	4	1	QQYAYSPLT	9													
JH35	3-21	3-10	1	6	11	LLTWFGESIVDV	12	IgG1	1-6	4	6	LQDYNYPLT	10	/	/	/	/	/								
JH38	3-7	2-2	2	3	25	QYCTRTTCYTDAFDM	15	IgG2	1-39	2	24	QKSCGVPT	8	/	/	/	/	/								
JH39#	3-30	3-22	2	3	26	GADTSGYSALDG	12	IgG1	1-39	3	16	QQSYSPPT	9													
JH40#	4-31	3-9	2	4	27	EARYYDLLTDSYSGGFDDQ	18	IgG1	3-20	1	11	QQYGSPPRT	9													
JH55	3-30	3-3	3	5	21	DGVQYFDIWSYSHNWFDV	20	IgG1	3-20	5	12	QHYGSTPPT	10	/	/	/	+	+	/							
JH56#	1-8	3-22	2	5	14	SLYYDRREYAOWFDS	15	IgG1	3-20	4	16	QQYGRSPLT	9													
JH57	4-59	/	/	5	20	FSVAGWFDV	9	IgG2	1-39	2	10	QQSYSTLGYT	10	/	/	/	/	/								
JH60	3-23	3-10	1	5	11	SAGLLWFGNGNWFDV	15	IgG2	3-20	1	5	QQYGLSPST	9	+	+	+	+	/								
JH65	1-18	5-12	3	3	21	DRRKDTVATLGEIDAFDV	18	IgG1	1-39	1	5	QQTFSPRT	8	/	/	/	/	/								
JH66	3-48	/	/	3	11	DKLPVSDAFDI	11	IgG1	1-39	4	7	QQSYSIPLT	9	/	/	/	/	/								
JH69	3-7	2-15	2	1	7	GGGFLIEH	8	IgG2	4-1	5	10	HQFYCAPLT	9	/	/	/	/	/								
JH76#	4-31	5-51/18	3	6	ND	GLDPTMAYGMDV	12	IgG1	1-39	4	9	QQSYSHPN	8													
JH78#	1-8	/	/	5	13	GITWGWFDV	9	IgG2	3-15	3	10	QQYNDWPRPT	9													
JH81#	7-4	3-10	1	4	11	DRRGVLGELLYYFDY	15	IgG1	1-39	2	7	QQSYITPV	9													
JH82#	3-48	6-13	2	3	14	TYSASWYRGDAFDI	14	IgG1	1-9	1	5	QQLNSYPLWT	10													
JH96	3-30	2-2	2	4	22	YNLEYCAGSICAFDR	16	IgG1	3-15	2	5	QQYNDWPRPT	9	/	/	/	/	/								
JH101#	1-69	/	/	4	25	DGSWINHRPSPFDY	14	IgG1	3-20	2	7	QVYGNQSVHT	9													
JH103	4-31	/	/	5	27	YQDDEAIPRWFDA	13	IgG3	2-28	5	15	MQALQLPIT	9	/	/	/	/	/								
JH113	3-33	/	/	4	24	GSQGDYVPFDY	11	IgG1	1-5	1	15	QQYSTYAWT	9	/	/	/	+	/	/							
JH114#	1-46	/	/	4	14	DGDGTAYSGY	10	IgG1	3-20	1	9	QQYGNLSLWT	9													
JH117#	3-23	3-22	2	4	14	STNYDSTGYAAGYYFEF	19	IgG1	3-20	1	14	QQYGSSE	8													
JH124#	4-39	6-19	2	5	ND	LKYSDDVWHETGFCQ	14	IgG1	1-39	4	6	QQYSAPLT	9													
JH125#	3-9	3-16	3	4	15	RVIGAKYFDR	10	IgG2	1-12	4	7	QQTNTFPLT	9													
JH129	1-18	3-10	3	6	15	SVRGVFMNLNYDYGYMDV	18	IgG1	1-33	4	9	QQYDNLVPT	9	/	/	/	/	/								
JH131#	3-7	/	/	4	17	TDITNCGYFDS	11	IgG1	1-5	1	7	QQYNSYSLT	9													
JH132	4-39	2-15	3	4	14	QAVAAAIPIVYFDY	15	IgG1	1-39	5	17	HQTYGFPST	9	/	/	/	+	+	+							
JH135	1-18	3-22	2	3	16	DRQYLDLRGDLVLAFAFI	18	IgG1	1-39	1	2	QQSFSTPGT	9	/	/	/	/	/								
JH147#	3-30	5-12	2	6	9	PWRRGYSGYDNDVLDV	16	IgG1	3-20	2	3	QQYASSMYT	9													
JH148	3-23	/	/	5	17	NEGEYDYNWFDV	12	IgG1	1-5	1	5	QQSHYSRT	9	/	/	/	/	/								
JH154	3-33	3-9	2	6	7	APGDYDILAEYYFYKMDV	19	IgG1	3-15	2	6	QQYNNWPYT	9	/	+	+	+	+								
JH156	3-7	3-3	1	5	20	DKARLRFLEPSNWFDV	16	IgG2	1-27	5	12	QKYDRAPHS	9	/	/	/	/	/								
JH160#	3-21	3-9	1	4	25	FLRLYLEWPHTDY	13	IgG1	3-20	4	9	HQYGFSSR	8													
JH162	3-15	5-24	2	4	11	DGDVYNPFAY	10	IgG1	1-33	2	9	QQYDLSLPS	9	/	/	/	/	/								
JH165	3-7	2-2	2	4	21	VQCTRTSCWGLMFFDH	16	IgG2	4-1	4	18	QQYDFPLT	9	/	/	/	/	/								
JH167#	4-59	/	/	6	11	LTPPSFYSGMDV	12	IgG2	3-20	2	4	QQYGSSTGYT	10													
JH179	3-23	6-19	2	2	21	VHASAWYLKWFDDL	14	IgG3	4-1	2	14	QQYSSTPYT	9	+	+	+	+	+								
JH183#	3-11	3-16	2	4	11	DWGRGPD	7	IgG2	3-20	1	11	QYYGPRRRWT	10													
JH186	1-2	3-10	2	4	9	GNYYGSGSDYYFDY	14	IgG1	1-39	4	14	QQSYGNPS	8	+	+	+	+	/								
JH188	3-30	/	/	5	10	CVGANCPNWFDV	12	IgG1	1-17	1	0	LQHNSYPWT	9	+	+	+	+	/								
JH192	3-48	3-22	2	4	21	GPPYSY	6	IgG1	3-15	4	5	HQYNDWPLT	10	/	/	/	/	/								
JH194	7-4	/	/	4	21	VQLGSRDFRADL	12	IgG1	1-5	1	17	QQYDNYSWT	9	/	/	/	+	/								
JH195	4-34	5-24	2	6	21	QDGYNYGGGYYFYGMVDV	18	IgG1	3-20	1	11	QQCGSLPMT	9	/	/	/	/	/								
gmJH	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	V _k	J _k	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2								
JH16	4-39	3-22	2	4	5	HWYDSSGYPAGRY	14	IgG1	1-47	2	6	AAWDDSLSAVV	11	/	/	/	/	/								
JH21	3-15	3-3	3	4	21	SFGVIISDFDY	12	IgG1	1-44	1	9	AAWDVSLRGPV	11	/	/	/	+	/								
JH28#	3-74	2-21	3	3	16	LPFIVLMTEDASDI	14	IgG1	2-23	1	13	CSYAGTNTYV	10													
JH42	7-4	/	/	4	16	GTLNPPHFDY	10	IgG1	1-51	2	4	ATWDSSLSIVV	11	/	/	/	/	/								
JH48#	5-51	2-15	2	4	15	RGYCGDVACFSVDF	15	IgG1	1-40	2	9	QSYDSSLSGVV	11													
JH50	4-31	/	/	5	41	NIAPGRFDP	9	IgG2	2-23	3	19	CSYGGSFNWL	10	/	/	/	+	/								
JH59#	1-2	/	/	6	7	APQTPYYYGLAV	13	IgG3	1-44	3	10	AAWDDSLNGWV	11													
JH63	4-34	/	/	4	33	GHVPRD	6	IgG3	1-47	3	16	AAWDDNLSGFWV	12	+	+	+	+	+								
JH75	4-34	/	/	4	4	GYPTNDEEHSKGRGPPFYFDY	23	IgG2	3-21	2	2	QVSDNTSDHPVV	12	/	/	/	/	/								
JH79	4-31	/	/	4	13	IPDFNKYHFD	11	IgG1	2-14	2	7	SSYTTTSTLVI	11	/	/	/	/	/								
JH88	3-66	/	/	6	14	LKDWRWGHYSYYGMDV	17	IgG1	1-44	2	7	AAWDDSLTGPV	11	/	/	/	/	+								
JH91	3-21	6-19	2	4	16	VFSAWAFDF	9	IgG1	2-8	1	7	TSYAGNDFV	10	/	/	/	/	/								
JH93	4-39	1-1/20	2	4	15	HTWGAWNDRPFYFDY	16	IgG1	3-1	2	13	QAWDSSSTHVV	10	/	/	/	/	/								
JH95	4-59	4-17	2	5	7	LSRENYDDYALFDP	14	IgG2	1-47	3	6	AAWDDSLSGPM	11	/	/	/	/	/								
JH105#	4-4	3-16	2	4	40	ESYRSANHLWGTYQYVGIDY	20	IgG3	2-8	2	ND	SSLAVSDNLI	10													
JH107#	3-23	4-23	2	4	19	PIVPHGGNSGLDC	13	IgG2	2-14	2	ND	SSYTSTTL	9													
JH109#	5-51	1-1/20	2	5	8	QLNWNDFTGNNRDSGWFDV	19	IgG1	2-14	2	16	SSHISGSTLVV	11													
JH128	4-59	1-1/20	2	4	26	LNWNEFRFDH	10	IgG1	6-57	3	14	QSYHVRDWW	9	/	/	/	/	+								
JH143#	5-51	/	/	6	22	AAYDSVWVSGNYYYGMDV	20	IgG3	2-23	2	16	CSWAGSRNPVV	11													
JH171	4-39	4-17	3	3	22	HGVTTDIAKPEIRDAFDF	18	IgG2	2-23	1	8	CSFAGYGTWV	10	/	/	/	/	/								
JH185#	4-59	6-13	2	5	20	MRSDTWFLNWFDV	14	ND	2-14	2	8	TSYTSNTLVI	9													

Table 10: Ig gene usage and features of antibodies from B cells expressing kappa and lambda light chain

Ig	HEAVY						LIGHT						REACTIVITY					
	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Length	SC	Vic%	Jc%	V-Mut.	CDR3 (aa)	Length	ss	ds	LPS	ins.	HEp2
gm169-3κ	3-48	3-3	2	6	40	EDDFWGSVYQTDYRFYPVDV	20	IgG1	3-20	1	0	QQYGGSSPWT	9	/	/	/	/	/
gm169-3λ									1-47	2	15	AAWDDSLSGVV	11	/	/	/	/	/
gm169-16κ	1-8	/	/	6	8	RGSTVYYYYYGMVDV	14	IgG1	1-33	5	0	QQYDNLSS	8	/	/	/	/	/
gm169-16λ#									2-11	1	ND	CAYAGDHKWV	10					
gm169-20κ	3-7	3-22	2	4	6	APPGSGSEYYFDY	13	IgG2	4-1	2	4	HOYYSSPYT	9	/	/	/	/	/
gm169-20λ									3-21	3	2	QVWDDSSDHPH	11	/	/	/	/	/
gm169-22κ	3-23	2-2/15or3-22	2	4	22	DSCGSPTCRTRYHSFDY	17	IgG1	3-15	2	6	QQYNRWPPHT	10	/	/	/	/	+
gm169-22λ									3-21	2	15	QVWDGIVV	8	/	/	/	/	/
gm169-24κ	4-4	3-3	2	5	14	GRDYDFWSGYSRQKEMYNWFD	22	IgG3	2-28	2	3	MQALQTPYT	9	+	+	+	+	+
gm169-24λ									9-49	1	1	GADHGSGSNFVVV	13	+	+	+	+	+
gm169-28κ	3-33	1-26	3	1	27	GNRLGATTFFH	11	IgG3	1-5	2	6	QQYKSLNT	8	+	+	+	+	+
gm169-28λ									2-14	3	5	SSFTSSSTLAWV	12	+	+	+	+	+
gm169-31κ	3-66	3-22	1	3	8	NGGYDSSGYWQRWDAFDI	19	IgG1	1-39	1	1	QQSYSTPWT	9	/	/	/	/	/
gm169-31λ									3-25	2	1	QSADSSGTSVV	11	/	/	/	/	/
gm169-34κ#	4-4	2-15	2	3	15	ATEKYCRGGSCYFHDAFDI	19	IgG1	1-39	1	8	QQSYSTPRT	9					
gm169-34λ									1-40	1	6	QSYDSSLSGVY	11	+	+	+	+	+
gm169-38κ	3-23	3-22	2	4	3	NIVNYDSSGPLY	14	IgG1	3-15	1	6	QQYNNWPRT	9	/	/	/	/	/
gm169-38λ									3-21	3	1	QVWDDSSDHQV	11	/	/	/	/	/
gm169-64κ	3-7	6-19	2	2	4	DYGLSYSSVFSINWYFDL	19	IgG3	3-20	5	4	QQYGGSPIT	9	/	/	/	/	+
gm169-64λ									1-40	3	1	QSYDSSLGGSV	11	+	+	+	+	/
gm169-70κ	3-13	3-10	3	4	1	GVMVRGVITYYFDY	14	IgG1	1-39	5	0	QQSYSTLIT	9	/	/	/	+	/
gm169-70λ#									1-40	3	0	QSYDSSLSGVW	11					
gm169-76κ	3-11	/	/	6	2	DTPGRLVHYYYMDV	14	IgG1	2-28	2	6	VQALQTPYT	9	/	/	/	/	/
gm169-76λ									2-11	2	0	CSYAGSYTLV	10	/	/	/	/	/
gm169-80κ	4-39	1-26	2	5	14	DLGGSSSFRF	10	IgG3	2-28	2	10	MQALQTPYT	9	+	+	+	+	+
gm169-80λ									3-21	2	5	QVWDDSSDHHVV	12	/	/	/	/	/
gp169-26κ	3-33	6-19	2	4	25	DRDSSGLYFLDY	12	IgG1	4-1	5	8	QQYSSVT	8	/	/	/	/	/
gp169-26λ									1-44	3	6	AAWDDSLNGPV	11	/	/	/	/	/
gp169-53κ	4-4	/	/	4	6	AQVYFDY	7	IgG1	4-1	3	16	QHYYSSPST	9	/	/	/	/	/
gp169-53λ									2-14	3	10	CSYTNSTTLGV	11	/	/	/	/	/
gp169-64κ	3-30	2-2	2	4	11	DWGGYCSSTSCSYFDY	16	IgG1	3-11	3	2	QQRFWHWPRGIT	11	/	/	/	+	+
gp169-64λ									6-57	2	2	QSYDSSNVV	10	/	/	/	/	/
gp169-103κ	4-39	3-9	1	5	25	VFRYFDWLWGFDP	13	IgG1	4-1	5	11	QQYFSTPVT	9	+	+	+	+	+
gp169-103λ									6-57	3	2	QSYDSSNVV	9	/	/	/	/	+
gp169-127κ	1-69	/	/	4	9	DTGGGYFDY	9	IgG1	3-11	2	1	QQRSNWPPYT	10	+	+	+	/	/
gp169-127λ									1-51	2	0	GTWDDSLSEGV	11	/	/	/	/	/
gp169-144κ	4-4	1-26	2	5	17	GPGSGNYFGGYDNWFD	17	IgG2	1-6	5	9	LDYNYPFT	9	/	/	/	+	/
gp169-144λ#									2-8	3	5	SSYAGSFWV	9					
174-120κ	1-24	/	/	3	5	VTEGGRFDAFDI	12	IgG1	2-30	2	0	MQGTHWPMYT	10	/	/	/	/	/
174-120λ									2-14	3	5	SSYTSSSTWV	10	/	/	/	/	/
176-650κ	3-9	1-26	2	5	6	DSSVYSGRYFGIDS	14	IgG3	4-1	4	5	QQYYSTPRLT	10	/	/	/	/	/
176-650λ#									4-69	2	ND	QTWGTGIYV	10					
HW-174κ	7-81	6-13	2	6	2	THRLYNNSWFPLVYDMDV	18	IgG1	3-20	1	ND	QQYGGSPRT	9	+	+	+	+	+
HW-174λ#									2-14	1	3	SSYXTSTYV	10					

#, antibody was not expressed/tested; gm, IgG⁺ memory B cell; gp, IgG⁺ plasmablast; RF, DH gene reading frame; V-Mut., number of V gene mutations from FWR1 to CDR2, inclusively; SC, IgG isotype subclass; ss, ssDNA; ds, dsDNA; ins., insulin; +, reactive in ELISA/IFA; /, non-reactive in ELISA/IFA; ND, not determined; HW, previously published healthy control

Table 11: Ig gene usage, CDR3 aminoacid sequence and reactivity of mutated and respective unmutated germline IgG memory B cell antibodies from SLE patients after reversion of somatic mutations

Ig	HEAVY							LIGHT							REACTIVITY (mut)		REACTIVITY (rev)	
	VH	D	RF	JH	V-Mut.	CDR3 (aa)	Reverted CDR3 (aa)	V κ / λ	J κ / λ	V-Mut.	CDR3 (aa)	Reverted CDR3 (aa)	poly	HEp2	poly	HEp2		
gm169-8	4-34	2-15	1	6	16	GPRNSQLPLDYFYYMDV	GPRNSQLLLDYYYYYMDV	λ 1-47	2	14	AAWDDSLSGVV	AAWDDSLSGVV	+	+	+	+		
gm169-28 λ	3-33	1-26	3	1	27	GNRLGATTFHH	ENRLGATTFQH	λ 2-14	3	5	SSFTSSSTLAWV	SSYTSSSTLAWV	+	/	/	/		
gm169-61	4-39	/	/	6	12	QEYTTSVYYYYMDV	QEYTTSVYYYYMDV	κ 3-20	4	8	QQYGSSPLT	QQYGSSPLT	+	+	/	/		
174-5	1-69	4-11/4	3	4	15	GLTTVTRFDY	GLTTVTRFDY	κ 3-11	1	11	QQRSNWPPWT	QQRSNWPPWT	+	+	+	+		
174-33	3-21	2-2	2	4	11	VRLGYCSNIRCAENKNFDF	VRLGYCSSTSCAENKNFDY	λ 1-40	3	6	QSYDNKLRRV	QSYDSSLRRV	+	+	/	/		
174-45	3-53	6-19	3	4	16	VLVQWLVPNGYFDY	VLVQWLVPNGYFDY	λ 2-11	1	8	CSYAGTYTYV	CSYAGSYTYV	/	/	+	+		
174-54	4-59	2-15	2	2	13	RGTLGSGTGAHCATDWYFDL	RGTLGYSGGSCATDWYFDL	λ 3-12	6	9	QAWDSSSDHPGV	QVWDSSSDHPGV	+	+	/	+		
174-67	3-30	4-23	3	6	2	DLTTVDPSYYYYGMDV	DLTTVDPSYYYYGMDV	κ 1-6	1	0	LQDYNYPWT	LQDYNYPWT	/	+	/	/		
174-82	5-51	4-23	2	4	19	THDGNFFWDY	THGGNFFWDY	λ 2-14	1	8	SSYTRTNTYTYV	SSYTSSSTLTYY	/	+	/	/		
174-87	3-48	6-13	2	4	1	VGRTSPLYSSSWYGFY	VGRTSPLYSSSWYGFY	κ 3-20	3	0	QQYGSSPFT	QQYGSSPFT	/	+	/	/		
174-119	3-48	2-2	2	4	7	GGYCTSTSCHEPLFGVVMTPRYFDY	GGYCSSTSCHEPLFGVVMTPRYFDY	κ 3-20	5	2	QQYGSSPT	QQYGSSPT	/	/	/	/		
174-130	4-31	5-12	2	4	1	EGVYSAYPSAY	EGVYSGYPSDY	κ 3-11	3	1	QQRSNWPSFT	QQRSNWPSFT	+	+	/	/		
174-133	1-69	6-13	3	6	21	GIVAADYYGMDV	GIVAADYYGMDV	κ 2-28	2	4	MQSLQTSFT	MQALQTSYT	/	/	/	/		
174-156	4-39	2-2	2	4	14	RRQRGPDCSSTSCYFDY	RRQRGPDCSSTSCYFDY	κ 1-5	1	4	QHYDDFA	QQYNSYA	/	/	/	/		
174-174	4-39	4-17	2	5	13	REDYGDYGWDFS	REDYGDYGWDFS	λ 2-23	3	10	CSYAGRSIM	CSYAGSSTM	/	+	/	/		
175-29	5-51	7-27	3	4	29	LPLTGGACDY	LPLTGGACDY	κ 3-15	1	14	QQYNKWWT	QQYNNWWT	+	+	/	/		
175-77	3-23	6-19	3	4	13	PASVAGQGGVDY	PASVAGQGGVDY	κ 1-5	1	9	QQYNTYFQWT	QQYNSYSQWT	+	/	/	/		
175-107	3-7	1-20/7	3	2	20	TARIAGTGGGYFDV	TARITGTGGWYFDL	κ 1-5	2	13	QQYKSYT	QQYNSYT	/	+	/	+		
175-128	3-23	3-10	3	4	36	DGRRGFLRLVDYLD	DGRRGVLRLVDYFDY	κ 2-28	1	12	MQALQTPPT	MQALQTPPT	/	+	+	+		
175-140	4-59	6-19	3	5	18	DRAVAAMYNWVDL	DRAVAAMYNWFDS	κ 1D-43	4	24	HQYYGTPLT	QQYYSTPLT	+	+	+	+		
175-162	3-33	2-2	3	4	26	GQWGNWRENAVARHYFDF	GQWGNWRDIVARHYFDFY	κ 1-5	1	16	QQYNSSSPT	QQYNSYSPT	/	+	/	/		
175-165	3-23	5-18/5	1	3	10	DPPRWMLWTTDAFDI	DPPRWIQLWTTDAFDV	λ 1-44	1	2	ATWDDSLNGRV	AAWDDSLNGRV	/	/	/	+		
175-250	1-2	1-26	3	4	22	DRAIVGAANDFY	DRAIVGAANYFDY	κ 3-20	1	8	QCCGSST	QQYGSSPT	/	/	/	/		
175-264	3-33	6-19	2	1	14	DGGAYSSGWYPQH	DGGAYSSGWYPQH	κ 3-15	2	11	QQYNVRPYT	QQYNNWPYT	+	+	/	/		
175-276	1-24	3-10	3	5	22	PYFSTSGSLGDLFDP	PYYYGSGSLGDLFDS	κ 2-40	1	14	KQRLDGVT	MQRIDGVT	/	+	/	/		
175-277	3-21	6-19/13	3	6	3	PVTPGIALYGVDV	PVTPGIALYGMDV	κ 1-5	2	3	QQYNSDST	QQYNSYST	/	/	/	/		
175-295	3-9	6-13	2	4	3	GRLRSSTWGYFDY	GRLRSSWGYFDY	λ 2-23	3	3	CSYAGSSTWV	CSYAGSSTWV	/	+	/	/		

reverted amino acids in CDR3s are in bold; RF, reading frame; V-Mut., number of V gene mutations from FWR1 to CDR2, inclusively; mut, mutated antibody; rev, reverted antibody; +, reactive in ELISA/IFA; /, non-reactive in ELISA/IFA; poly, polyreactive to ss/dsDNA, LPS, and insulin; HEp2, reactive in HEp2 ELISA and/or IFA

Table 12: List of standard primers

Forward Primer	5' - 3' sequence
5' L-VH 1	ACAGGTGCCCACTCCCAGGTGCAG
5' L-VH 3	AAGGTGTCCAGTGTGARGTGCAG
5' L-VH 4/6	CCCAGATGGGTCCTGTCCCAGGTGCAG
5' L-VH 5	CAAGGAGTCTGTTCCGAGGTGCAG
5' Agel VH1	CTGCA <u>ACCGGT</u> GTACATTCCCAGGTGCAGCTGGTGCAG
5' Agel VH1/5	CTGCA <u>ACCGGT</u> GTACATTCCGAGGTGCAGCTGGTGCAG
5' Agel VH3	CTGCA <u>ACCGGT</u> GTACATTCTGAGGTGCAGCTGGTGGAG
5' Agel VH3-23	CTGCA <u>ACCGGT</u> GTACATTCTGAGGTGCAGCTGTTGGAG
5' Agel VH4	CTGCA <u>ACCGGT</u> GTACATTCCCAGGTGCAGCTGCAGGAG
5' Agel VH 4-34	CTGCA <u>ACCGGT</u> GTACATTCCCAGGTGCAGCTACAGCAGTG
5' Agel VH 1-18	CTGCA <u>ACCGGT</u> GTACATTCCCAGGTTGAGCTGGTGCAG
5' Agel VH 1-24	CTGCA <u>ACCGGT</u> GTACATTCCCAGGTCCAGCTGGTACAG
5' Agel VH3-33	CTGCA <u>ACCGGT</u> GTACATTCTCAGGTGCAGCTGGTGGAG
5' Agel VH 3-9	CTGCA <u>ACCGGT</u> GTACATTCTGAAGTGCAGCTGGTGGAG
5' Agel VH4-39	CTGCA <u>ACCGGT</u> GTACATTCCCAGCTGCAGCTGCAGGAG
5' Agel VH 6-1	CTGCA <u>ACCGGT</u> GTACATTCCCAGGTACAGCTGCAGCAG
5' L V _K 1/2	ATGAGGSTCCCYGCTCAGCTGCTGG
5' L V _K 3	CTCTTCCTCCTGCTACTCTGGCTCCCAG
5' L V _K 4	ATTTCTCTGTTGCTCTGGATCTCTG
5' Pan V _K	ATGACCCAGWCTCCABYCWCCCTG
5' Agel V _K 1-5	CTGCA <u>ACCGGT</u> GTACATTCTGACATCCAGATGACCCAGTC
5' Agel V _K 1-9	TTGTGCTGCA <u>ACCGGT</u> GTACATTCAGACATCCAGTTGACCCAGTCT

SUPPLEMENTAL INFORMATION

5' AgeI V _κ 1D-43	CTGCA <u>ACCGGT</u> GTACATTGTGCCATCCGGATGACCCAGTC
5' AgeI V _κ 2-24	CTGCA <u>ACCGGT</u> GTACATGGGGATATTGTGATGACCCAGAC
5' AgeI V _κ 2-28	CTGCA <u>ACCGGT</u> GTACATGGGGATATTGTGATGACTCAGTC
5' AgeI V _κ 2-30	CTGCA <u>ACCGGT</u> GTACATGGGGATGTTGTGATGACTCAGTC
5' Age V _κ 3-11	TTGTGCTGCA <u>ACCGGT</u> GTACATTCAGAAATTGTGTTGACACAGTC
5' Age V _κ 3-15	CTGCA <u>ACCGGT</u> GTACATTCAGAAATAGTGATGACGCAGTC
5' Age V _κ 3-20	TTGTGCTGCA <u>ACCGGT</u> GTACATTCAGAAATTGTGTTGACGCAGTCT
5' Age V _κ 4-1	CTGCA <u>ACCGGT</u> GTACATTCGGACATCGTGATGACCCAGTC
5' L V _λ 1	GGTCCTGGGCCCAGTCTGTGCTG
5' L V _λ 2	GGTCCTGGGCCCAGTCTGCCCTG
5' L V _λ 3	GCTCTGTGACCTCCTATGAGCTG
5' L V _λ 4/5	GGTCTCTCTCSCAGCYTGTGCTG
5' L V _λ 6	GTTCTTGGGCCAATTTTATGCTG
5' L V _λ 7	GGTCCAATTCYCAGGCTGTGGTG
5' L V _λ 8	GAGTGGATTCTCAGACTGTGGTG
5' AgeI V _λ 1	CTGCT <u>ACCGGT</u> TCCTGGGCCCAGTCTGTGCTGACKCAG
5' AgeI V _λ 2	CTGCT <u>ACCGGT</u> TCCTGGGCCCAGTCTGCCCTGACTCAG
5' AgeI V _λ 3	CTGCT <u>ACCGGT</u> TCTGTGACCTCCTATGAGCTGACWCAG
5' AgeI V _λ 4/5	CTGCT <u>ACCGGT</u> TCTCTCTCSCAGCYTGTGCTGACTCA
5' AgeI V _λ 6	CTGCT <u>ACCGGT</u> TCTTGGGCCAATTTTATGCTGACTCAG
5' AgeI V _λ 7/8	CTGCT <u>ACCGGT</u> TCCAATTCYCAGRCTGTGGTGACYCAG
5' Ab sense	GCTTCGTTAGAACGCGGCTAC

Reverse Primer**5' - 3' sequence**

3' C γ CH1	GGAAGGTGTGCACGCCGCTGGTC
3' IgG (internal)	GTTCGGGGAAGTAGTCCTTGAC
3' Sall JH 1/2/4/5	TGCGAAGT <u>CGAC</u> GCTGAGGAGACGGTGACCAG
3' Sall JH 3	TGCGAAGT <u>CGAC</u> GCTGAAGAGACGGTGACCATTG
3' Sall JH 6	TGCGAAGT <u>CGAC</u> GCTGAGGAGACGGTGACCGTG
3' C κ 543	GTTTCTCGTAGTCTGCTTTGCTCA
3' C κ 494	GTGCTGTCCTTGCTGTCCTGCT
3' BsiWI J κ 1/4	GCCAC <u>CGTACG</u> TTTGATYTCCACCTTGGTC
3' BsiWI J κ 2	GCCAC <u>CGTACG</u> TTTGATCTCCAGCTTGGTC
3' BsiWI J κ 3	GCCAC <u>CGTACG</u> TTTGATATCCACTTTGGTC
3' BsiWI J κ 5	GCCAC <u>CGTACG</u> TTTAATCTCCAGTCGTGTC
3' C λ	CACCAGTGTGGCCTTGTTGGCTTG
3' XhoI C λ	CTCCTCA <u>CTCGAG</u> GGYGGGAACAGAGTG

Restriction sites are underlined

Supplemental materials

Antibodies and ELISA reagents

ELISA capture antibody

goat anti-human IgG, Fc γ -specific

Jackson ImmunoResearch Laboratories
Suffolk, Great Britain

ELISA secondary antibody

Anti-human IgG, Fc γ (HRP) goat

Jackson ImmunoResearch Laboratories
Suffolk, Great Britain

ELISA standard and control antibodies

Human IgG1, kappa

Sigma Aldrich Chemie GmbH
Steinheim, Germany

ED38 (high positive polyreactive Ig)

Meffre, 2004

JB40 (low positive polyreactive Ig)

Wardemann, 2003

mGO53 (non positive polyreactive Ig)

FACS

anti-human CD19 (APC)

Invitrogen Co
Carlsbad, CA, USA

anti-human IgG (Biotin)

Invitrogen Co
Carlsbad, CA, USA

anti-human CD27 (FITC)

Invitrogen Co
Carlsbad, CA, USA

anti human CD38 (PE)

Invitrogen Co
Carlsbad, CA, USA

Streptavidin (PECy7)

Invitrogen Co
Carlsbad, CA, USA

Antigens for ELISA

DNA sodium salt from salmon testes

Sigma Aldrich Chemie GmbH
Steinheim, Germany

Insulin solution, human

Sigma Aldrich Chemie GmbH
Steinheim, Germany

LPS, E.coli 055:B5

Sigma Aldrich Chemie GmbH
Steinheim, Germany

ELISA plates, pre-coated

QUANTA Lite™ ANA ELISA

INOVA Diagnostics Inc.
San Diego, CA, USA

SUPPLEMENTAL INFORMATION

Anti-Ro	ORGENTEC Diagnostika GmbH Mainz, Germany
Anti-La	ORGENTEC Diagnostika GmbH Mainz, Germany

Bacteria and vectors

<i>E. coli</i> DH10B	Clontech, Paolo Alto, CA, USA
Vectors (with constant regions of IgH, Igκ and Igλ chain)	Kind gift of Dr. J. Ravetch Rockefeller University 1230 York Avenue New York, NY 10021, USA

Cell line

HEK 293T	Invitrogen GmbH Karlsruhe, Germany
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Chemicals, buffer and solutions

2x HBSS (HEPES buffered saline solution), pH=7,05	280 mM NaCl 50 mM HEPES 10 mM KCl 12 mM dextrose 1,5 mM Na ₂ HPO ₄ · 7H ₂ O
50x TAE (Tris Acetate EDTA) buffer	2 M Tris-Base 0,05 % (v/v) acetic acid 0,05 M EDTA (pH=8)
5x loading buffer	60% (w/v) sucrose 1mM cresol red
Ammoniumchloride (NH ₄ Cl)	Sigma Aldrich Chemie GmbH Steinheim, Germany
Calciumchloride (CaCl ₂)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Chloroquine (C ₁₈ H ₂₆ ClN ₃)	Sigma Aldrich Chemie GmbH Steinheim, Germany
Cresol red (C ₂₁ H ₁₇ O ₅ SNa)	Acros Organics Geel, Belgium
Dextrose (C ₆ H ₁₂ O ₆ · H ₂ O)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
DTT (Dithiothreitol)	Invitrogen GmbH Karlsruhe, Germany

SUPPLEMENTAL INFORMATION

EDTA ($C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$, ethylenediamine tetraacetic acid)	Sigma Aldrich Chemie GmbH Steinheim, Germany
ELISA blocking solution	1x PBS 0,05 % (v/v) Tween [®] 20 1 mM EDTA
Ficoll- Paque PLUS [™]	GE Healthcare Bio-Sciences AB, Uppsala, Sweden
Fluoromount-G	SouthernBiotech Birmingham, AL, USA
GIBCO [™] 10x PBS	Invitrogen GmbH Karlsruhe, Germany
Glycerol ($C_3H_8O_3$)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Glycine ($C_2H_5NO_2$)	Sigma Aldrich Chemie GmbH Steinheim, Germany
HEPES ($C_8H_{18}N_2O_4S$, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Igepal CA-630	Sigma Aldrich
LB-Agar	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
LB- Medium	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Lysis buffer	0.01 M DTT 8U RNasin 4U Prime RNase Inhibitor in 0.5x nuclease free PBS
Potassium bicarbonate ($KHCO_3$)	Sigma Aldrich Chemie GmbH Steinheim, Germany
Potassium chloride (KCl)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Potassium phosphate (KH_2PO_4)	Sigma Aldrich Chemie GmbH Steinheim, Germany
RosetteSep [®]	Stemcell Technologies Inc., Vancouver, BC, Canada
Sodium azide (NaN_3)	Sigma Aldrich Chemie GmbH Steinheim, Germany
Sodium chloride (NaCl)	Sigma Aldrich Chemie GmbH Steinheim, Germany
Sodium phosphate ($NaH_2PO_4 \cdot 7H_2O$)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Sucrose ($C_{12}H_{22}O_{11}$)	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Terrific-Broth	Carl Roth GmbH + Co. KG, Karlsruhe, Germany

SUPPLEMENTAL INFORMATION

Trizma[®] base

Sigma Aldrich Chemie GmbH
Steinheim, Germany

Tween[®] 20

Carl Roth GmbH + Co. KG,
Karlsruhe, Germany

Reverse transcription

RHP mix

0.016 % NP-40
1 µl 10 % DTT
165 ng RHP
3 U PRIME RNase Inhibitor

RT mix

2.15x RT-Puffer
14.3 mM DTT
1.8 mM dNTPs each
50 U Superscript III
4 U RNasin
3 U PRIME RNase Inhibitor

Kits employed

HiSpeed[®] Plasmid Maxi Kit

Qiagen GmbH
Hilden, Germany

HRP Substrate Kit

Bio-Rad Laboratories GmbH,
München, Germany

QIAPrep[®] Spin Miniprep Kit

Qiagen GmbH
Hilden, Germany

QIAquick[®] gel extraction Kit

Qiagen GmbH
Hilden, Germany

QIAvac[®] 96 DNA purification Kit

Qiagen GmbH
Hilden, Germany

Immunofluorescence assay

BIOS GmbH,
München, Germany

INNO-LIA ANA Update

Innogenetics Biologicals,
Gent, Belgium

Enzymes and additives

Restriction enzymes

AgeI, BsiWI, Sall, XhoI

New England Biolabs GmbH
Frankfurt am Main, Germany

RT (Reverse transcription)- and PCR

10x PCR buffer	Qiagen GmbH Hilden, Germany
5x first strand buffer	Invitrogen GmbH Karlsruhe, Germany
100 mM dNTP Set (dATP, dCTP, dGTP, dTTP), PCR grade	Invitrogen GmbH Karlsruhe, Germany
HotStar Taq DNA Polymerase	Qiagen GmbH Hilden, Germany
Nonidet P40	Sigma Aldrich Chemie GmbH Steinheim, Germany
Nuclease-free water	Eppendorf AG Hamburg, Germany
RNase Inhibitor 5 PRIME	Eppendorf AG Hamburg, Germany
RNasin®	Promega GmbH Mannheim, Germany
Seakem® LE Agarose	Cambrex Bio Science Rockland, Inc. Rockland, ME, USA
Superscript™ III RT	Invitrogen GmbH Karlsruhe, Germany
Taq DNA Polymerase	Roche Diagnostics GmbH Mannheim, Germany

Cloning

T4 DNA Ligase	New England Biolabs GmbH Frankfurt am Main, Germany
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ELISA

1-Step™ ABTS	Pierce Biotechnology, Inc. Rockford, IL, USA
Horseradish Peroxidase Substrate Kit	BIO-RAD Laboratories GmbH Munich, Germany

Antibody purification

Protein G Sepharose™ 4 Fast Flow	GE Healthcare Bio-Sciences AB Uppsala, Sweden
Bio-Spin chromatography columns	BIO-RAD Laboratories GmbH Munich, Germany

Equipment and lab material

1.5 ml reaction tubes	SARSTEDT AG & Co. Nümbrecht, Germany
96well Multiply- PCR plate	SARSTEDT AG & Co. Nümbrecht, Germany
96well PCR plate	Eppendorf AG Hamburg, Germany
Alpha Imager	Alpha Innotech Corporation San Leandro, CA, USA
Axioplan 2 imaging fluorescence microscope	Carl Zeiss MicroImaging GmbH, Jena, Germany
BioPhotometer	Eppendorf AG Hamburg, Germany
BioSpin [®] chromatography columns	Bio-Rad Laboratories GmbH, München, Germany
Cell culture dishes (150 mm)	Falcon, Becton Dickinson GmbH Heidelberg, Germany
Cellstar [®] sterile serological pipettes 2 ml, 5 ml, 10 ml, 25 ml, 50 ml	Greiner Bio-One Frickenhäusen, Germany
Centrifuge 5180R (Rotor A-4-81) <i>Used for reaction tubes larger than 2 ml</i>	Eppendorf AG Hamburg, Germany
Centrifuge 5417R (Rotor F-45-30-11) <i>Used for reaction tubes up to 2 ml</i>	Eppendorf AG Hamburg, Germany
CO ₂ incubator CB210	Binder GmbH Tuttlingen, Germany
Clean bench HERAsafe KS12	Thermo Electron Corp. Langenbold, Germany
Domed 12-Cap Strips (PCR Tubes Strips)	Bio-Rad Laboratories GmbH, München, Germany
ELISA high binding microtiter plates (96well, flat bottom)	Costar, Corning B.V Schiphol-Rijk, Netherlands
FACSVantage	BD Biosciences Franklin Lakes, NJ, USA
Incubator BE 600	Memmert GmbH + Co.KG, Schwabach, Germany
Inoculation loops, inoculation needles (polystyrene)	Nunc GmbH & Co. KG Wiesbaden, Germany
Mastercycler [®] ep gradient	Eppendorf AG Hamburg, Germany
Microseal 'F' Film	Bio-Rad Laboratories GmbH, München, Germany

SUPPLEMENTAL INFORMATION

Microwave	Panasonic Germany Hamburg, Germany
Multichannel Pipet-Lite® with LTS 2...20 µl, 20...200 µl, 100...1200 µl	Rainin Instrument, LLC Woburn, MA, USA
Multipette plus	Eppendorf AG Hamburg, Germany
Parafilm	Pechiney Plastic Packaging, Chicago, IL, USA
Petri dishes (100 mm)	Greiner Bio-One Frickenhausen, Germany
Pipetboy acu	INTEGRA Biosciences GmbH Fernwald, Germany
Pipet-Lite® with LTS 0.1...2 µl, 2...20 µl, 20...200 µl, 100...1000 µl	Rainin Instrument, LLC Woburn, MA, USA
Polypropylene tubes (15 ml, 50 ml)	SARSTEDT AG & Co. Nümbrecht, Germany
Rotator drive STR4, Stuart scientific	Keison Products Essex, England
SpectraMax 190 Microplate Reader	Molecular Devices Corporation Sunnyvale, CA, USA
Thermomixer comfort	Eppendorf AG Hamburg, Germany
Vaccu-Pette/96	Scienceware/Bel-Art Pequannock, NJ, USA
Vortex genie 2	Scientific Industries, Inc. Bohemia, NY, USA
Water bath with thermostat	JULABO Labortechnik GmbH Seelbach, Germany
Water bath GFL 1008	GFL Gesellschaft für Labortechnik Burgwedel, Germany

Media and supplements for bacterial and tissue culture

Ampicillin	Sigma Aldrich Chemie GmbH Steinheim, Germany
GIBCO™ Antibiotic-Antimycotic (100X)	Invitrogen GmbH Karlsruhe, Germany
GIBCO™ DMEM (Dulbecco's Modified Eagle Medium)	Invitrogen GmbH Karlsruhe, Germany
GIBCO™ L-Glutamine	Invitrogen GmbH Karlsruhe, Germany
GIBCO™ Trypsin	Invitrogen GmbH Karlsruhe, Germany

SUPPLEMENTAL INFORMATION

GIBCO™ FBS (fetal bovine serum)	Invitrogen GmbH Karlsruhe, Germany
LB-Medium	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
LB-Agar	Carl Roth GmbH + Co. KG, Karlsruhe, Germany
Nutridoma-SP	Roche Diagnostics GmbH Mannheim, Germany
Terrific-Broth-Medium	Carl Roth GmbH + Co. KG, Karlsruhe, Germany

Nucleotides and nucleic acids

100 bp DNA Ladder	New England Biolabs GmbH Frankfurt am Main, Germany
1 kb DNA Ladder	New England Biolabs GmbH Frankfurt am Main, Germany
dNTPs (Desoxyribonucleotidyl Triphosphate)	Invitrogen GmbH Karlsruhe, Germany
Oligonucleotides	MWG-BIOTECH AG, Ebersberg, Germany
Random Hexamer Primer pd(N) ₆	Roche Diagnostics GmbH Mannheim, Germany

Software

Adobe Illustrator CS3	Adobe Systems GmbH München, Germany
GraphPad Prism 5	GraphPad Software, Inc. San Diego, CA, USA
SeqMan™ II	DNASTAR, Inc. Madison, WI, USA
SoftMax Pro 5	Molecular Devices GmbH Ismaning, Germany

Internet sources

IMGT	http://imgt.cines.fr/
IgBlast	http://www.ncbi.nlm.nih.gov/igblast/

APPENDIX

Acknowledgments

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Publications

Publications in Journals

Mietzner B, Tsuiji M, Scheid J, Velinzon K, Tiller T, Abraham K, Gonzalez JB, Pascual V, Stichweh D, Wardemann H, Nussenzweig MC. (2008) Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors.. *Proc Natl Acad Sci USA.* , 105:9727-32.

Schulz TJ, Thierbach R, Voigt A, Drewes G, Mietzner B, Steinberg P, Pfeiffer AF, Ristow M. (2006) Induction of Oxidative Metabolism by Mitochondrial Frataxin inhibits Cancer Growth: Otto Warburg revisited. *J Biol Chem.* , 281:977-81.

Thierbach R, Schulz TJ, Isken F, Voigt A, Mietzner B, Drewes G, von Kleist-Retzow JC, Wiesner RJ, Magnuson MA, Puccio H, Pfeiffer AF, Steinberg P, Ristow M. (2005) Targeted Disruption of Hepatic Frataxin Expression causes impaired Mitochondrial Function, decreased Life Span and Tumor Growth in Mice. *Hum Mol Genet.* , 14:3857-64.

Publications at Meetings

Mietzner B, Tsuiji M, Scheid J, Velinzon K, Tiller T, Abraham K, Gonzalez JB, Pascual V, Stichweh D, Nussenzweig MC, Wardemann H. Autoreactive IgG memory antibodies in patients with systemic lupus erythematosus arise from nonreactive and polyreactive precursors.

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Mietzner B, Tsuiji M, Scheid J, Feraoun N, Yurasov S, Velinzon K, Wardemann H, Nussenzweig MC. IgG plasmablast and memory B cell antibodies in patients with SLE.

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SELBSTSTÄNDIGKEITSERKLÄRUNG

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Ich versichere, dass diese Arbeit in dieser oder anderer Form noch keiner anderen Prüfungsbehörde vorgelegt wurde. Der Inhalt der Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät I der Humboldt Universität zu Berlin vom 1.09.2005 ist mir bekannt.

Berlin, den 20. Juli 2009

Brun Henning Mietzner